INVESTIGATING THE PHYTOTOXICITY OF OIL SAND TAILINGS WATER FORMED DURING ATMOSPHERIC FINES DRYING PROCESSING

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Masters of Science

in the Toxicology Graduate Program

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Jessica Louise Cutter

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Chair of the Toxicology Graduate Program

Toxicology Centre

University of Saskatchewan

44 Campus Drive

Saskatoon, Saskatchewan, Canada, S7N 5B3

ABSTRACT

Oil sands operators are being faced with the challenge of reclaiming the large volumes of slurry tailings created during oil sands processing. New regulations mandate that operators must minimize fluid tailings by capturing fines in dedicated disposal areas, leading to a 'trafficable' or solid deposit. Adding a polyacrylamide polymer to the tailings and thinly spreading them over a sloped disposal area (a process developed by Shell Canada Energy known as the atmospheric fines drying or AFD process) has been shown to enhance the dewatering of tailings which leads to a dry deposit at a much faster rate than traditional methods.

Hydroponic experiments using the emergent aquatic macrophytes cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) were conducted to investigate the phytotoxicity of waters formed during AFD processing. The phytotoxicity of AFD release waters was compared to the phytotoxicity of traditional mature fine tailings (MFT) reclaim water through the monitoring of plant water uptake and whole plant fresh weight over the course of the experiment. It was found that there are no significant differences between the phytotoxicity observed in the MFT and AFD treatments and it was also found that spring runoff melt water from the AFD deposits is less phytotoxic than the original release water.

Two additional hydroponic studies using cattail and common reed were also conducted. The first examined the phytotoxic effects attributable solely to the naphthenic acids isolated from Shell's Muskeg River Mine tailings, and the second evaluated the phytotoxic effects of amending mature fine tailings with gypsum. It was found that the gypsum amended tailings caused greater phytotoxicity in cattail and common reed than

tailings without gypsum added. Furthermore, both species were tolerant to growing in nutrient media spiked with naphthenic acids (40 mg/L).

The phytotoxicity experiments conducted also demonstrated that common reed is consistently more tolerant to growing in water associated with oil sands tailings and is therefore the more appropriate choice for use in reclamation strategies involving wetland plants.

Mass spectrometry was used to determine the naphthenic acid molecular profiles for Shell oil sands tailings. Using low resolution mass spectrometry, no detectable features or changes to the composition of naphthenic acids attributable to Shell processing were found. High-resolution mass spectrometry provided insight into possible plant mediated changes and biodegradation of naphthenic acids. It appears as though, to some extent, cattail is able to dissipate naphthenic acids, which could explain the susceptibility of cattail to the phytotoxic effects of naphthenic acids. Further research is required to determine whether the changes observed in the naphthenic acid mixture are due to microbial degradation and/or a phytotoxic response of the plants studied.

ACKNOWLEDGMENTS

I would like to thank my supervisors Dr. John Headley and Dr. Jim Germida for their guidance and support during the completion of this project. I would also like to thank my advisory committee, Dr. Steven Siciliano, Dr. Sarah Hughes, and Dr. Karen Tanino, my external examiner. I'd like to give a very special thank you to Dr. Hughes, who throughout my journey through graduate studies was constantly there to share guidance, expertise, words of encouragement, and a laugh when one was needed most. I would also like to thank Kerry Peru and Dr. Brian Fahlman for their advice and technical expertise. In addition, I'd like to thank Dr. Jeff Schoenau and Dr. Richard Farrell for providing me with space in the Phytotron to conduct my experiments.

I am grateful for the assistance provided throughout the years by the faculty, students, and staff at the Toxicology Centre. To my colleagues at the National Hydrology Research Centre, thank you for your support and friendship.

Funding for this project was provided by the Natural Sciences and Engineering Research Council through an Industrial Post Graduate Scholarship, Shell Canada Energy, and also by Natural Resource Canada (Dr. Randy Mikula of CanmetENERGY).

Finally, I would like to thank my family. Mum and Dad, my sisters Nicky, Kelly, and Stacey – thank you for laughing at my science jokes, listening to my endless stories, and for encouraging me.

This thesis is dedicated to Holly – who is always ready with kind words, support, encouragement, laughter, and love. Thank you for everything.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFD - atmospheric fines drying

cm - centimeter

CT - consolidated tailings

m^3 - cubic meters

°C - degrees Celsius

DNA - deoxyribonucleic acid

ESI-MS - electrospray ionization mass spectrometry

ERCB - Energy Resources Conservation Board

g - gram

ha - hectares

MFT - mature fine tailings

µg/L - microgram per liter

µm - micrometer

mg/L - milligram per liter

mg/ml - milligram per milliliter

mL - milliliter

mL/min - milliliter per minute

mM - millimolar

M - molar concentration

MRM - Muskeg River Mine

km^2 - square kilometers

m^2 - square meters

L - liter

LC-MS - liquid chromatography-mass spectrometry

nM - nanometer

OSPW - oil sands process-affected water

psi - pounds per square inch

RSD - relative standard deviation

SRC - Saskatchewan Research Council

NaOH - sodium hydroxide

SPE - solid phase extraction

H_2SO_4 - sulfuric acid

USEPA - United States Environmental Protection Agency

%wt - weight percentage

%wt/wt - weight solute per weight total solution

CHAPTER 1

INTRODUCTION

1.1 Oil sands mining and tailings

One of the world's largest natural oil sands deposits covers an area of approximately 140,000 km² in northern Alberta, Canada, stretching over the Athabasca, Cold Lake, and Peace River regions [Johnson & Miyanishi, 2008] (Figure 1.1). It is estimated that these deposits contain approximately 2.5 trillion barrels of recoverable bitumen in the form of oil sand [Penner & Foght, 2010]. The Athabasca oil sands deposit alone (42,000 km²) contains approximately 173 billion barrels of bitumen available for recovery with current technology [Barrow *et al*., 2010]. Bitumen is defined as an extra-heavy oil that does not naturally flow to a well [American Association of Petroleum Geologists, 2011]. Bitumen is a heavy, carbon rich, hydrogen-poor hydrocarbon that is upgraded to synthetic crude through the removal of carbon and sulphur and the addition of hydrogen [Johnson & Miyanishi, 2008]. Due to the high concentrations of nitrogen, sulphur, oxygen, and heavy metals within bitumen, the costs for extraction, transportation, and refining are greater for oil sands crude than those for conventional crude oil [American Association of Petroleum Geologists, 2011].

The Athabasca deposit is an example of an unconventional oil deposit whereby the oil is extracted from the ground using techniques other than the traditional well method. Traditional well methods include steam assisted gravity drainage and cyclic steam stimulation. The methods utilizing steam require large amounts of energy and water in order to produce the steam required to mobilize the oil [American Association of Petroleum Geologists, 2011].

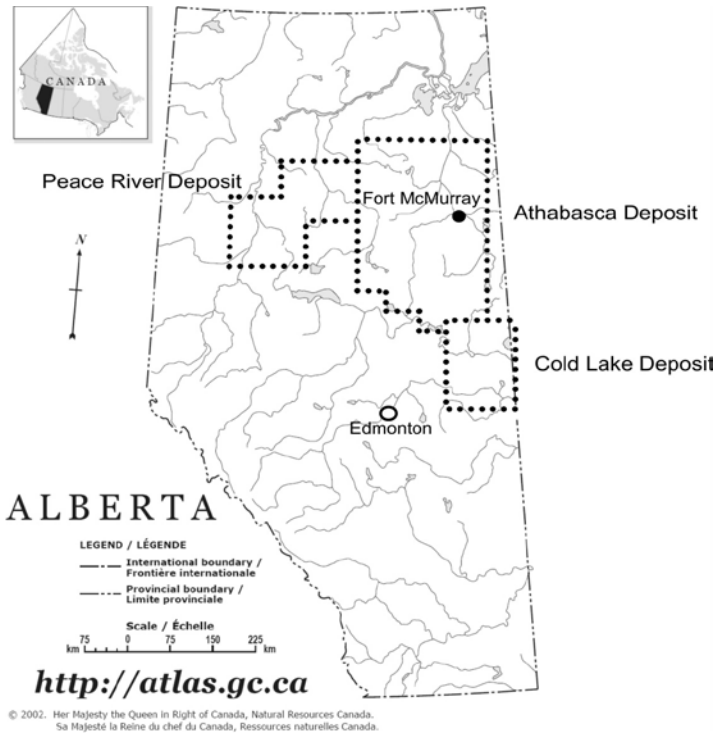

Figure 1.1. Map of Alberta, Canada, illustrating the Peace River, Athabasca, and Cold Lake oil sand deposits [Allen, 2008].

Surface mining is one of the methods used to access unconventional oil such as that contained within oil sand deposits [Rooney & Bayley, 2011]. The oil sand in the Athabasca deposit is a mixture of clay or shale, bitumen, sand, and water [Brough *et al*., 2010]. Surface mining involves the removal of trees, peat, and overburden that lie on top of the oil sand deposit [Johnson & Miyanishi, 2008]. Large machinery is then used to remove the oil sand from the open mine and the oil sand is then transported to a processing facility. Typically, a caustic hot water process is used to extract oil from the surface mined oil sands where caustic soda is used to separate bitumen from sand, clay, organics, and dissolved metals [Rogers *et al*., 2002]. Approximately 2 m^3 of water is required for the extraction of every 1 m^3 of oil produced [Mikula *et al*., 2008] and it is

estimated that more than 3 m^3 of tailings are produced (following water recycling) per 1 m^3 of bitumen produced from oil sand [Xu *et al.*, 2008]. To reduce pressure on the fresh water supply, 75% of the water required for processing must come from recycled process water [Long *et al*., 2005]. The Alberta Environmental Protection and Enhancement Act [1993] currently holds oil sands companies to a zero-discharge policy, therefore oil sands tailings are discharged into large tailings ponds for storage and water recycling. These tailings ponds presently cover approximately 180 km^2 [Kasperski & Mikula, 2011].

Mature fine tailings (MFT) are formed in tailings ponds when coarse sands settle rapidly, leaving a suspension of residual bitumen and fines in water. Gravity settling of the fines releases a layer of water at the surface of the tailings pond that is collected for re-use [Penner & Foght, 2010]. After two to three years of settling, MFT contain approximately 30% by weight solids that are trapped in the water [Wang *et al*., 2010] and it is estimated that without further treatment, additional consolidation of MFT could take hundreds of years [Johnson & Miyanishi, 2008]. The current estimate is that there is between 720 - 1000 million m^3 of MFT being stored within the surface mining region of Alberta (Athabasca Deposit, Figure 1.1) with approximately 1.5 m^3 of MFT formed for every 1 m^3 of bitumen produced [Kasperski & Mikula, 2011].

Efforts have been made to find alternatives to 'liquid' tailings ponds. Many of these efforts include the addition of settling aids to flocculate and/or coagulate fines, which enhance the dewatering of the tailings, producing a dry deposit [Hazma *et al*., 1996; Long *et al*., 2005; Yuan & Shaw, 2007; Wang *et al*., 2010]. The stacking of these dewatered tailings in mined-out pits is being investigated as an integral part of the reclamation strategy [Xu *et al*., 2008].

In 2009, as part of a larger initiative to regulate tailings management, the Energy Resources Conservation Board (ERCB) introduced Directive 074. The directive details performance criteria for the decrease of fluid tailings and the formation of trafficable deposits [ERCB, 2009]. Directive 074 is the result of oil sands operators being unable to meet the fines capturing targets outlined in the original development applications [American Association of Petroleum Geologists, 2011]. Operators are required to reduce fluid tailings through fines (mineral solids with particle size equal to or less than 40 μm) captured in dedicated disposal areas [Energy Resources Conservation Board, 2009] with the intention of returning the water released from the fines back into the extraction process. Oil sands mining operators are therefore being challenged to convert the vast amount of liquid tailings into 'trafficable' deposits which are defined as deposits which can support heavy machinery. The MFT accumulated in tailings ponds is a reclamation problem because the material is unable to bear loads sufficient for the reclamation of ponds to dry landscapes [Demoz & Mikula, 2012].

1.2 Tailings reclamation

1.2.1 The atmospheric fines drying (AFD) process

In order to meet the requirements set forth by Directive 074, Shell Canada Energy has developed the atmospheric fines drying (AFD) process (Figures 1.2 and 1.3). The AFD process involves the in-pipe addition of an anionic polyacrylamide polymer to MFT. The polyacrylamide polymer acts as a flocculent, enabling the fines suspended in the liquid portion of the tailings to form larger particles, which are able to settle, 'releasing' the water from the MFT. The flocculated tailings are subsequently thinly spread on a sloped

disposal area (called a ‘cell’) and allowed to rapidly dewater. Water released from the tailings (termed ‘release water’) is collected in weirs positioned at the base of each cell, and this water is returned to the processing facility for re-use. Once sufficiently dry, the materials are transferred to waste disposal areas such as mined-out pits. The long-term intention is to cap these pits with overburden [Shell Canada Energy, 2010].

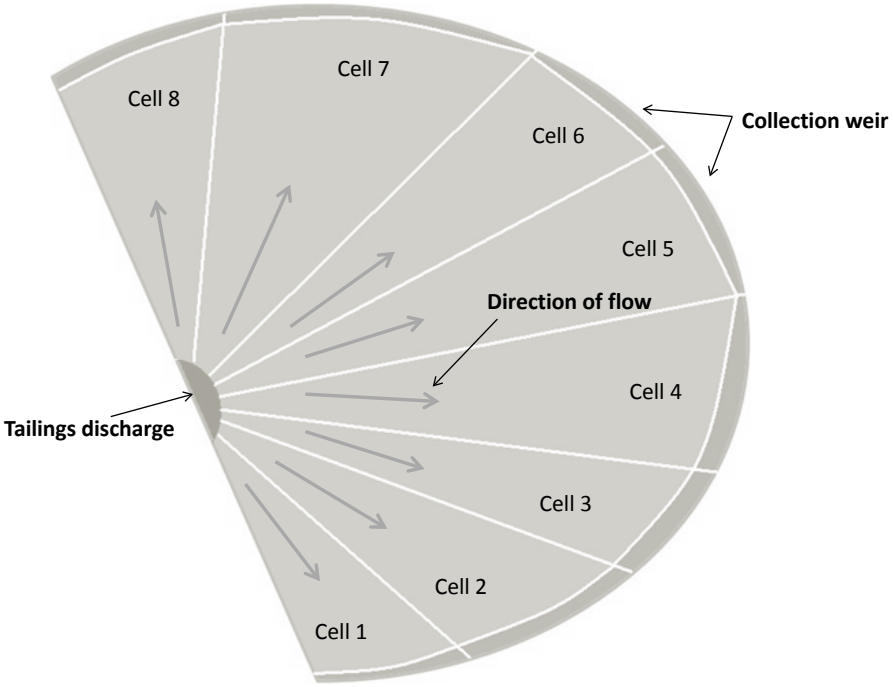

Figure 1.2. Schematic representation of Shell Canada Energy’s atmospheric fines drying (AFD) pilot site located at the Muskeg River Mine, Fort McMurray, Alberta, Canada.

Figure 1.3. Aerial photograph of Shell Canada Energy's atmospheric fines drying (AFD) pilot site at the Muskeg River Mine Site, Fort McMurray, Alberta, Canada. Access roads can be seen between each set of two cells. [Photo from Radio Netherlands Worldwide, 2012].

1.2.2 Consolidated tailings (CT)

Consolidated tailings (CT) are formed when an inorganic coagulant such as gypsum (calcium sulphate) is added to MFT. Consolidated MFT is pumped into tailings ponds where the mixture is allowed to settle and the sand and fines settle out together. The coagulant causes the fine particles suspended in MFT to clump together, which then settle, and the water that is released can be collected and re-used in oil sands processing. Following approximately one year, the material is composed of 70% to 80% by weight solids [Kasperski & Mikula, 2011].

1.2.3 Wetland plants as part of the reclamation strategy

As part of the licensing agreement with the Alberta Government, oil sands operators are required to reclaim land disturbed by oil sand mining; this includes integrating wetlands into the reclaimed landscape. Wetlands are vital components of the landscape that provide habitat for fish and wildlife species, increasing landscape diversity. Wetlands also protect and improve the quality of ground and surface water by providing flood control and controlling water and wind erosion of soil [Oil Sands Wetlands Working Group, 2000]. Current guidelines recommend that 33% of the reclaimed oil sands landscape be comprised of wetlands [Alberta Environment, 2007] which will total 66,000 ha [Trites & Bayley, 2009].

Cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) are native perennial wetland plants that are ideal for reclamation of oil sands tailings due to their tolerance of moderate salinity. Cattail is a common emergent macrophyte found in freshwater wetland areas [Rogers *et al*., 1998]. Cattail are tolerant of a broad range of climatic conditions [Mitich, 2000], reduced soil conditions, persistent flooding, and are aggressive invaders in brackish salt water marshes and freshwater wetlands [United States Department of Agriculture, 2010]. The recommended optimal growing pH for cattail is in the range of 5.5 – 7.5, which demonstrates the ability of cattail to grow in acidic to basic soil and water [United States Department of Agriculture, 2010]. Cattail regenerate through rhizome growth and during the first year rhizomes can spread up to 2 m in diameter. Following two growing seasons, a colony of cattails can cover an area of over 50 m^2 [Mitich, 2000].

Common reed is frost resistant and can survive in temperatures as cold as -20°C. Common reed is well adapted to grow in low-lying wet areas such as fresh and saltwater marshes and swamps [Mal & Narine, 2004; United States Department of Agriculture, 2010]. Common reed is also able to grow in alkaline conditions similar to those found in tailings ponds [Mal & Narine, 2004], and the optimal pH for common reed growth is in the range of 4.8 to 8.2 [United States Department of Agriculture, 2010].

The transpiration rates of both cattail and common reed are very high [Mitich, 2000; Mal & Narine, 2004], and when coupled with their tolerance of a range of water quality conditions, these two species are an ideal choice to investigate for use in oil sand reclamation efforts.

1.3 The phytotoxicity of oil sand tailings

The effects of oil sands tailings on birds, fish, and small aquatic organisms have been well studied; however, the same level of research has not been conducted on the phytotoxicity of oil sands tailings. In the past ten to fifteen years, there has been a gradual increase in the number of studies examining the phytotoxic effects of oil sands tailings and this is likely due to an increase in the focus of the reclamation of the oil sands mining landscape. Depending on the form of tailings material, plant species, and plant life cycle stage evaluated, the results of phytotoxicity studies vary. For example, Crowe *et al*. [2001] found that wetland plants exposed to oil sands tailings had higher photosynthetic rates and it was concluded that cattail and clover (*Trifolium hybridum* L.) are able to adapt to growth in oil sands effluent. Crowe *et al*. [2002] also evaluated the effects of oil sands tailings on terrestrial plant growth and concluded that the germination of species

such as rye, wheat, pea, canary grass, and clover is inhibited when exposed to tailings materials. Research conducted by Renault *et al.* has established that there are suitable woody plant species such as red-osier dogwood (*Cornus sericea* L.), buffalo berry (*Sheperdia canadensis* (L.) Nutt), and willow (*Salix* sp.) that possess a high tolerance to oil sands tailings materials [Renault *et al*., 1998; 1999; 2000]. Grasses, more specifically altai wildrye (*Elymus angustus* (Trin.) Plig.) and slender wheatgrass (*Agropyron trachycaulum* (Link) Malte ex H.F. Lewis), have also been found to tolerate the high salinity and high pH of oil sands tailings [Renault *et al*., 2004]. The following sections will describe in more detail the major constituents of oil sands tailings and the potential phytotoxicity associated with each.

1.3.1 The phytotoxicity of naphthenic acids

Naphthenic acids are compounds naturally present in bitumen that are released during mining and extraction processes [Armstrong *et al*., 2009] and they represent one source of toxicity in oil sands tailings ponds [Allen, 2008]. Classically defined naphthenic acids are a group of alkyl-substituted cyclic and aliphatic carboxylic acid compounds [Allen, 2008] with the general chemical formula $C_nH_{2n+Z}O_2$ [Clemente & Fedorak, 2005].

The present definition of naphthenic acids includes not only the classically defined naphthenic acids (containing two oxygen atoms), but also the acid-extractable fraction (referred to as naphthenic acid fraction components) that includes aromatic components, with or without nitrogen and/or sulfur atoms [Headley *et al*., 2011] (Figure 1.4). Future reference to naphthenic acids within this text refers to the broader definition

(naphthenic acids fraction components) and is not restricted solely to the classically defined naphthenic acids. Naphthenic acids are non-volatile and chemically stable [Clemente & Fedorak, 2005] and act as surfactants [Apostol & Zwiazek, 2004]. It should also be noted that the concentration of naturally occuring naphthenic acids varies depending on the location of the oil deposit [Clemente & Fedorak, 2005].

Commercially prepared naphthenic acids have a variety of industrial uses. For example, naphthenic acids are used as preservatives and flame retardants in fabric and to prevent fungus growth in wood. Naphthenic acids are also used to improve water resistance and adhesion of concrete [Clemente & Fedorak, 2005]. Armstrong *et al*. [2008] found that the use of commercially prepared naphthenic acids in a phytotoxicity study caused phytotoxic effects in cattail, however naturally occuring naphthenic acids extracted from oil sands tailings did not exert significant phytotoxic effects. Armstrong concluded that caution must be taken when substituting commercially prepared naphthenic acids as surrogates for the naphthenic acids found in oil sands tailings [Armstrong *et al*., 2008].

Armstrong *et al*. [2008] evaluated the phytotoxicity of two naphthenic acid mixtures on the native emergent aquatic macrophytes cattail, common reed, and hard stem bulrush (*Schoenoplectus acutus* (Muhl. ex Bigelow) A. Love & D. Love). It was found that naphthenic acids alter water relations within the plants, which is manifested by changes in transpiration rates and ultimately plant growth. The pH of the tailings affects the ionization of naphthenic acids and it was found by Armstrong *et al*. [2009] that in tailings with an alkaline pH, naphthenic acids are found in their ionized water soluble form, and in tailings with an acidic pH, naphthenic acids are found in their non-ionized

form. In addition, Armstrong *et al.* determined that the non-ionized form of naphthenic acids is more phytotoxic than the ionized form – causing greater decreases in transpiration and plant growth [Armstrong *et al*., 2009].

In order to determine if the surfactant properties of naphthenic acids intensify the effects of sodium chloride in plant cells (e.g. membrane leakiness, inhibition of water uptake), Apostol and Zwiazek [2003] treated six-month old jack pine (*Pinus banksiana* Lamb.) seedlings with a solution of naphthenic acids (150 mg/L). The researchers found that naphthenic acids alone did not affect membrane leakiness in roots and needles but when coupled with increased salinity (45mM NaCl), naphthenic acids increased the electrolyte leakage from needles [Apostol & Zwiazek, 2003]. The concentration of naphthenic acids evaluated by Apostol and Zwiazek [2003] did not have a significant effect on the fresh weight of roots and shoots; however stomatal conductance was significantly reduced in plants treated with naphthenic acids.

1.3.1.1 Analytical detection of naphthenic acids

Due to the complexity of the mixture of organic acids defined as naphthenic acids, the precise characterization of naphthenic acids is difficult [Headley *et al*., 2009]. In the present study, low resolution analysis of naphthenic acids was performed using electrospray ionization mass spectrometry (ESI-MS) and high resolution analysis was performed on a LTQ Orbitrap Velos mass spectrometer using electrospray ionization in the negative ion mode. The instrument settings used for both the low and high-resolution analyses are reported in Section 6.2 and in Appendices B and C. In order to remove residual salts and to concentrate the polar organic constituents, prior to

analysis with low and high resolution mass spectrometry, tailings samples were cleaned-up using solid-phase extraction (SPE) columns (method reported in Section 6.2.2).

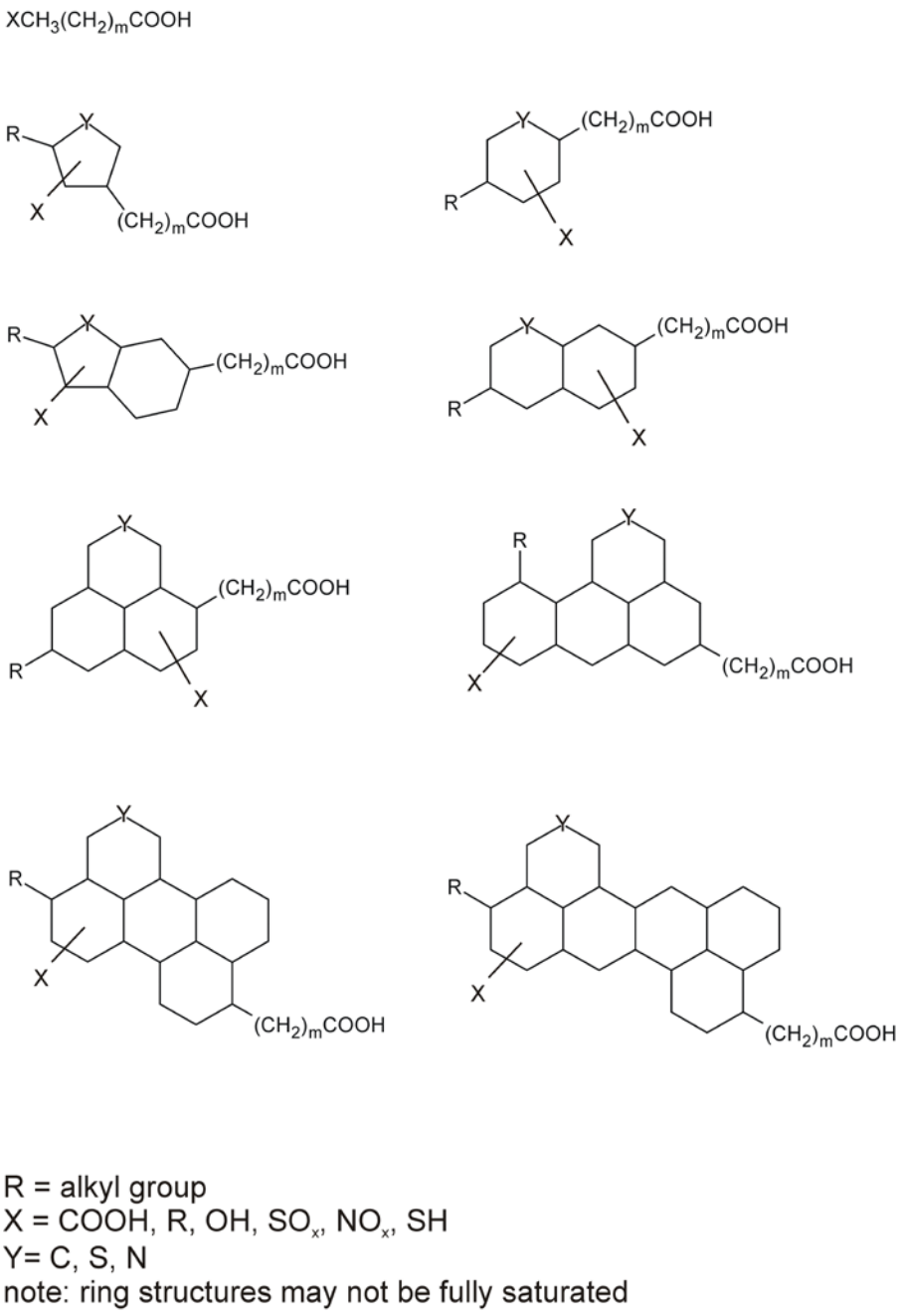

Figure 1.4. Representative molecular structures of naphthenic acid fraction components as outlined by the most recent naphthenic acid definition, which includes the classical naphthenic acids [Headley *et al*., 2011].

1.3.2 The phytotoxicity of high salinity

One of the major phytotoxic characteristics of oil sands tailings is high salinity. The large volumes of water discharged into ponds following processing contain elevated levels of sodium, sulphate, bicarbonate, and chloride [Renault *et al*., 1999]. Varying degrees of salinity exist in oil sands reclaimed wetlands [Trites & Bayley, 2009] with levels less than 1340 mg/L considered non-saline, less than 3350 mg/L slightly saline, and salinity above 2680 mg/L is observed to be detrimental to plants [Renault *et al*., 1998]. High concentrations of salts induce osmotic stress and salt accumulation in plants [Apostol & Zwiazek, 2003], and reduce stomatal conductance, which eventually leads to reduced plant growth [Renault *et al*., 1999]. Transpiration rates are affected by decreased root hydraulic conductivity, which in turn influences ion transport to the shoots of the plant [Apostol & Zwiazek, 2003].

Crowe *et al*. [2001] investigated the toxicity of oil sands tailings dike seepage to wetland plants and found an accumulation of stress related proteins in cattail roots that may be the result of osmotic stress caused by the high salinity of tailings. Apostol and Zwiazek [2003] found that a solution of 45mM NaCl caused a significant decrease in the fresh shoot weight, root respiration, and stomatal conductance in six-month old jack pine seedlings [Apostol & Zwiazek, 2003].

1.3.3 Phytotoxicity of consolidated tailings (CT)

Armstrong *et al*. [2010] conducted hydroponic phytotoxicity experiments to evaluate the phytotoxic effects of MFT amended with a 0.5% gypsum treatment using common reed. Gypsum was added to MFT and which was then mixed and left to settle

for one week. The amended tailings were then used in the hydroponic phytotoxicity experiments. It should be noted that all treatments evaluated induced phytotoxic effects in common reed however, Armstrong *et al*. [2010] found that of the different chemical treatments evaluated (MFT + 0.5% lime; MFT + 0.25% lime and 0.25% gypsum; MFT + 0.5% gypsum) MFT amended with 0.5% gypsum showed the least significant impact on the overall health of the common reed plants being evaluated.

Armstrong and colleagues [2010] evaluated the performance of common reed in simulated runoff and seepage waters from different MFT drying scenarios. The researchers also examined materials similar to AFD materials, where fine tailings were treated with a polymer or a combination of lime and gypsum and were then thinly spread to dry. Simulated runoff water was then collected and used in the hydroponic experiments. These waters were found to be phytotoxic, observed through reduced plant fresh weight and water uptake, and it was hypothesized to be a result of the combined effects of salinity, pH, and naphthenic acid concentrations [Armstrong *et al*., 2010].

Renault *et al*. [1998] evaluated the phytotoxic effects of the release water formed in association with the CT process using a variety of plant species native to the northern boreal forest surrounding the oil sands development [Renault *et al*., 1998]. The water that is released from the non-segregated tailings was collected and used in the hydroponic phytotoxicity experiments. At the conclusion of the four-week experiment, it was found that raspberry and strawberry seedlings were highly susceptible to the phytotoxic effects of the gypsum amended tailings (high mortality in the undiluted tailings water treatment group). Willow and aspen seedlings showed moderate phytotoxic effects (loss of leaves) and dogwood and a hybrid poplar species showed the highest tolerance. White and black

spruce, along with lodgepole pine had reduced rates of transpiration and some leaf tip necrosis. A similar experiment was conducted by Renault *et al*. [2003] to evaluate the ability of barley (*Hordeum vulgare* L.) to grow in soil irrigated with CT release water from tailings amended with gypsum. It was found that over the eight-week study period, barley plants were relatively tolerant to growing in soil irrigated with CT release waters containing gypsum, showing slight delays in germination, no effects on survival, and slight reduction in growth [Renault *et al*., 2003].

Redfield *et al*. [2003] evaluated the growth of red-osier dogwood (*Cornus sericea* L.) seedlings in reclamation soil bottom watered with Hoagland solution made with CT release water and gypsum CT watered with Hoagland solution made with gypsum CT release water [Redfield *et al*., 2003]. It was found that plants grown in Hoagland solution made from gypsum CT release water had significantly ($P < 0.001$) decreased survival rates, dry weights, and shoot heights compared to control plants. Plants grown in control soil watered with Hoagland solution made from gypsum CT release water showed 100% survival rates, and no significant differences ($P < 0.001$) in dry weight and shoot length when compared to control plants [Redfield *et al*., 2003].

Renault *et al.* [2004] also evaluated the phytotoxic effects of irrigating with release water from CT amended with gypsum on slender wheatgrass (*Agropyron trachycaulum* (Link) Malte ex H.F. Lewis) and altai wildrye (*Elymus angustus* (Trin.) Plig.). Experiments were conducted in a greenhouse and plants were grown in soil irrigated with CT release water. Germination was significantly reduced in slender wheatgrass grown in soil irrigated with gypsum amended CT release water. Once

successfully germinated, both species were able to survive and grow with little to no injury to the plants [Renault *et al*., 2004].

1.3.4 The Toxicity of polyacrylamide

In the AFD process being piloted by Shell Canada Energy, an anionic polyacrylamide polymer is added to MFT as an organic flocculent. Polyacrylamides are water soluble synthetic polymers of acrylamide or a combination of acrylamide and acrylic acid [Smith *et al*., 1996]. Due to their ability to flocculate soil particles, polyacrylamides are often used as soil additives to aid in soil erosion and runoff control [Krauth *et al*., 2008]. Polyacrylamides are able to bridge soil particles together through cation-bridging and Van der Waals forces, assisting in particle size enlargement to allow gravity settling of particles which limits soil transport via wind and water [Krauth *et al*., 2008]. Since polyacrylamides have the ability to flocculate fine particles suspended in the water column [Krauth *et al*., 2008], this technology is being investigated as a method to help dewater the large volume of oil sand tailings currently being stored in tailings ponds.

Barvenik [1994] reported that when applied at the concentrations used for agricultural purposes, water soluble anionic polyacrylamide has low toxicity to aquatic and terrestrial organisms. Polyacrylamide polymers are capable to travel only 0-20 cm from the point of application because they are irreversibly adsorbed to sediment and organic material [Lentz *et al*., 2008]. The real environmental concern lies with the small amount of residual acrylamide monomer (<0.05% wt/wt; leftover from production) that may be present in polyacrylamide polymers [Lentz *et al*., 2008]. Acrylamide is water soluble and is highly mobile in soil and groundwater [Friedman, 2003]. Although the acrylamide monomer has low toxicity to aquatic organisms it is a potential human

carcinogen and a known human and mammalian neurotoxin [Doerge *et al*., 2008; Lentz *et al*., 2008]. Rodent carcinogenicity studies examining dietary exposure to acrylamide and the formation of a DNA reactive metabolite (glycidamide) have led researchers to deem acrylamide a likely human carcinogen [Doerge *et al*., 2008]. Smith *et al*. [1996] conducted studies examining the environmental degradation of a polyacrylamide-thickening agent to acrylamide monomers. The findings of Smith *et al*. [1996] suggest that temperature and light can lead to the depolymerisation of polyacrylamide to acrylamide, however the conditions required for this depolymerisation are not environmentally realistic [Smith *et al*., 1996]. Polyacrylamide polymers are used as clarifiers in drinking water treatment [Lentz *et al*., 2008]. This application is closely regulated to ensure that the level of acrylamide monomer in drinking water does not exceed the United States Environmental Protection Agency (USEPA) drinking water guideline of 0.5 µg/L [Lentz *et al*., 2008]. Studies conducted using beans, corn, potatoes, and sugar beets grown in soil treated with polyacrylamide showed that any residual acrylamide absorbed into the plants was degraded after eighteen hours – the mechanism of the degradation within the plants is however unknown [Friedman, 2003].

1.4 Objectives

Using the aquatic macrophytes common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) and cattail (*Typha latifolia* L.), hydroponic experiments were conducted to fulfill the following research objectives:

1) To determine the phytotoxicity of release waters formed in connection with tailings produced by the atmospheric fines drying (AFD) process compared to reclaim water produced from traditional mature fine tailings (MFT).

2) To determine the effects of over wintering on AFD deposits by testing the phytotoxic effects of snowmelt runoff water from AFD deposits compared to similar waters produced from MFT deposits.

3) To determine the phytotoxicity of tailings treated with gypsum and hydroponic growth solution spiked with a naphthenic acid extract.

4) To determine the naphthenic acid molecular profile and fingerprint of AFD release and runoff waters in relation to MFT waters.

CHAPTER 2

THE PHYTOTOXICITY OF RELEASE WATERS FORMED IN CONNECTION WITH TAILINGS PRODUCED BY THE ATMOSPHERIC FINES DRYING PROCESS

2.1 Introduction

Approximately 2 m^3 of water is required for the extraction of every 1 m^3 of oil produced from oil sand [Headley *et al*., 2010] and it is estimated that more than 3 m^3 of tailings are produced (following water recycling) per 1 m^3 of bitumen produced from oil sand [Xu *et al*., 2008]. By the year 2020, it is expected that the total volume of liquid oil sands tailings stored within tailings ponds will exceed 1 billion m^3 [Grant *et al*., 2010]. New regulations (e.g. Directive 074) are mandating that operators must reduce fluid tailings by capturing fines in dedicated disposal areas in order to make a 'trafficable' or solid deposit [ERCB, 2009]. Due to the combination of the zero discharge policy and Directive 074, oil sands operators are faced with the challenge of reclaiming the large volumes of slurry tailings created during oil sands processing.

2.1.1 Atmospheric fines drying

In order to meet the regulatory demands, Shell Canada Energy has developed the atmospheric fines drying (AFD) process. This AFD technology is being piloted at the Muskeg River Mine near Fort McMurray, Alberta, Canada. The AFD process involves adding a polyacrylamide polymer to mature fine tailings (MFT) reclaim water. MFT refers to tailings containing approximately 30 %wt solids whereby following a period of

two to three years, the coarse sand fraction has settled while the fine solids and residual bitumen remain suspended and trapped in the water [Wang *et al*., 2010]. In the AFD process, the anionic polymer is used to flocculate the suspended fines in MFT, thereby increasing the efficiency of the settling process and hence water release. The polymer treated MFT reclaim water is then thinly spread over a sloped disposal area called a cell. Each cell is engineered with a slope ranging from 0.5% - 1.5% and is situated along a central tailings discharge to allow the spread of the tailings. Water released from the polymer treated tailings in a cell is termed 'release water'. The release water is captured weirs positioned at the base of each cell and this water is returned to the external tailings facility for re-use in the extraction process. The AFD process enhances the dewatering of tailings, which leads to a dry deposit at a much faster rate than traditional methods. The long-term intention is to stack these dewatered tailings layers in mined-out pits, leading to a trafficable deposit [Shell Canada Energy, 2010].

2.1.2 Phytotoxic constituents of oil sands tailings water

Previous studies were conducted that evaluated the effects of different oil sands reclamation strategies on fish [van den Heuvel *et al*., 1999; Nero *et al*., 2006; Lister *et al*., 2008], birds [Gurney *et al*., 2005; Gentes *et al*., 2007; Harms *et al*., 2010], phytoplankton [Leung *et al*., 2001], amphibians [Pollet & Bendell-Young, 2000; Hersikorn *et al*., 2010], and invertebrates [Farwell *et al*., 2009; Mackinnon *et al*., 2009]. However, there are few studies evaluating the effects of remediation strategies on wetland plants. Armstrong and co-researchers determined that common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) might be able to assist in the dewatering of oil sands tailings [Armstrong *et al*.,

2010]. It has also been determined that cattail (*Typha latifolia* L.) possess the ability to adapt to growing in oil sands tailings [Crowe *et al*., 2001]. Previous studies were also conducted on wetland plants using hydroponic nutrient media spiked with commercially prepared naphthenic acids and a laboratory prepared naphthenic acid extract from oil sands tailings [Armstrong *et al*., 2009]. Classically defined naphthenic acids are a complex mixture of organic acids [Quagraine *et al*., 2005] that are naturally present in bitumen and are released during the mining and extraction processes [Armstrong *et al*., 2009]. The current definition of naphthenic acids includes both the acid-extractable fraction that includes aromatic components, with or without nitrogen and/or sulfur atoms, as well as the classically defined naphthenic acids (containing two oxygen atoms [Headley *et al*., 2011]. Reference to naphthenic acids within this thesis refers to the broader definition of naphthenic acids and is not restricted to the classically defined naphthenic acids. Naphthenic acids have been identified to contribute to the toxicity of oil sands tailings [Allen, 2008] and due to the repeated recycling of oil sands water from tailings, over time they can become concentrated in tailings ponds [Quagraine *et al*., 2005]. The dissipation and phytotoxicity of two naphthenic acid mixtures was studied using the native emergent macrophytes cattail, common reed, and hard stem bulrush (*Scirpus acutus* (Muhl. ex Bigelow) A. Love & D. Love) [Armstrong *et al*., 2008]. Armstrong *et al*. [2008] found that naphthenic acids alter water relations within the plants, which was manifested by changes in transpiration rates and ultimately plant growth. It was also observed that the pH of the tailings water affects the ionization of naphthenic acids and in tailings water with an alkaline pH, naphthenic acids are ionized

and water soluble and were found to be less phytotoxic than when in their non-ionized form [Armstrong *et al*., 2009].

Using common reed, Armstrong *et al*. [2010] also evaluated the phytotoxicity of various oil sands tailings with chemical amendments, including a polyacrylamide polymer. It was found that when tailings amended with a polyacrylamide polymer were re-wetted with a simulated rainfall, subsequent runoff waters caused a significant reduction in fresh weight and water uptake in common reed [Armstrong *et al*. 2010].

Polyacrylamide polymers are used in mixtures with organic solvents to form thickening agents [Smith *et al*., 1997]. Phytotoxicity from polyacrylamide has not been previously reported [Smith *et al*., 1996]. However, there is concern that if polymers are degraded under environmental conditions, highly water-soluble and toxic acrylamides may be released into the surrounding environment [Smith *et al*., 1997] increasing the potential of surface and ground water contamination.

The benefits of using plants in conjunction with oil sands reclamation strategies consist of aiding in dewatering the tailings as well as providing wind and water erosion control [Renault *et al*., 2004]. As well, in many reclamation schemes, dried oil sands tailings will be capped and re-vegetated, providing wildlife habitat and aiding in stabilizing slopes [Johnson & Miyanishi, 2008]. It is therefore important to evaluate the ability of wetland plants to survive and grow in oil sands tailings affected water. The goal of the present study was to compare the relative phytotoxicity of the release water from the pilot AFD tailings treatment strategy to traditional MFT reclaim water and to evaluate the phytotoxicity of AFD tailings to wetland plants.

2.2 Materials and methods

2.2.1 AFD sample collection

MFT reclaim water and AFD release water was collected throughout the fall of 2010 at the AFD pilot site and was stored at 4ºC in 20 L non-leaching plastic pails until used in hydroponic studies in January of 2011. MFT reclaim water (200 L) was collected from the reclaim extraction point at the Muskeg River Mine external tailings facility (Fort McMurray, Alberta, Canada). MFT reclaim water is the water released from MFT once suspended fines have settled and it is collected from the surface of MFT ponds for re-use in oil sands processing. Collection of the AFD release water began once the flocculated MFT was deposited onto the AFD cells and continued daily for the first two to three weeks. Once the flow of release water from the deposit began to slow, sampling occurred every two to three days. The AFD release water was obtained by collecting 40 L samples from the weir at the base of each sloped AFD cell. The 40 L samples were pooled in cell-specific non-leaching plastic drums to form a combined sample of release water for each cell.

AFD release water samples investigated using the phytotoxicity assays include water collected from Cells 4, 7, and 8 because these cells were the only ones to formulate dry tailings. As the release water from Cells 4 and 7 was in short supply, a composite sample of Cells 4, 7, and 8 was investigated. Therefore, the tailings treatment groups for the phytotoxicity experiment were MFT reclaim water, Cell 8 release water, and a composite of Cells 4, 7, and 8. In summary, 60 L of each MFT, AFD Cell 8, and composite AFD release water were investigated in the phytotoxicity experiments.

2.2.2 Hydroponic experiments

Thirty day hydroponic experiments using the emergent macrophytes common reed and cattail were conducted following the methods described previously by Armstrong *et al*. [2010].

Cattail and common reed root cuttings were obtained in October 2010 from Bearberry Creek Water Gardens (Sundre, Alberta, Canada). Cuttings were mass cultured in plastic bins in quarter-strength modified Hoagland's nutrient medium (235 mg/L $Ca(NO_3)_2 \cdot 4H_2O$; 130 mg/L $MgSO_4 \cdot 7H_2O$; 165 mg/L KNO_3; 30 mg/L $NH_4H_2PO_4$; 17.5 mg/L Sequestrene 330 Fe (Fe-DTPA) with 0.1 mL of the following micronutrient solution per liter of media: 7 g/L H_3BO_3; 8.5 g/L $MnSO_4 \cdot H_2O$; 0.25 g/L $CuSO_4 \cdot 5H_2O$; 0.55 g/L $ZnSO_4 \cdot 7H_2O$; 0.25 g/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$; 1.25 g/L H_2SO_4) in a climate controlled environmental chamber for three weeks. Chamber conditions were maintained at a 16:8 hour, 25ºC:18ºC day/night cycle with an average light intensity of 162 $\mu mol/m^{-2}/s^{-1}$.

Once plants reached a shoot length of ~65 cm and ~70 cm for common reed and cattail respectively, they were transferred to individual 2.5 L amber glass jars (Figure 2.1) containing quarter-strength modified Hoagland's nutrient medium and were allowed to acclimatize for a period of two weeks. Jars were wrapped in aluminium foil to avoid excess algal growth in the test vessels. Each jar was provided with a glass aeration tube attached to a silicone aeration tube and aquarium air pumps were used to supply air and mixing to the media with each pump split to five separate jars using a five-way gang valve. A plastic foam plug was fitted into the opening of the jar, with a slit allowing

support to the plant, and a hole to support the glass aeration tube. The plastic foam plug also prevented excess evaporation of the growth medium.

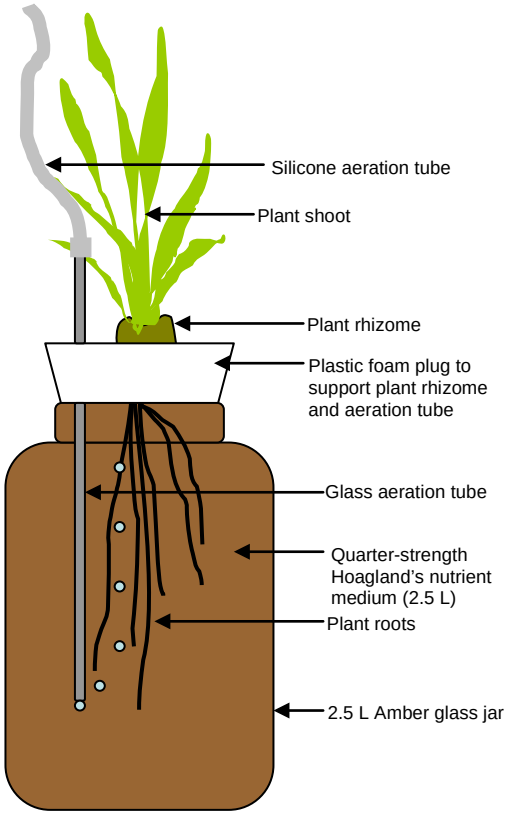

Figure 2.1. Testing unit used for aquatic plants during 30 day hydroponic experiments Diagram adapted from Armstrong [2008].

Following the two week acclimation period, plants were randomly assigned treatments (Day 0) and there were three replicates per treatment group for a total of 12 plants per species. Control plants were provided with a fresh 2.5 L volume of quarter-strength modified Hoagland's nutrient medium. For plants assigned tailings water treatment, the nutrient medium was replaced with 2.5 L of either AFD Cell 8 tailings

release water, AFD composite tailings release water, or MFT reclaim water. Prior to each use, MFT reclaim water and AFD release water was stirred to achieve a uniform mixture.

Over the 30 day experiment, water uptake was assessed by monitoring the volume of water taken up by the plants and jars were topped up with either MFT, oil sands release water, or control nutrient media, to the original 2.5 L volume every five days (on Days 5, 10, 15, 20, 25, and 30). In order to minimize the variability in water uptake data among replicates, water uptake data was normalized to starting fresh weight and is therefore reported as millilitres of water per gram starting fresh weight. Whole plant fresh weight was monitored by recording the mass of each plant on Day 0 and then again on Day 30. In order to reduce stress to the plants, fresh weight was only measured on the last day of the experiment and not throughout the entire course of the experiment. To further minimize handling the plants, shoot and root lengths were not monitored.

2.2.3 Data analysis

All data were tested for normality using the Shapiro-Wilk Test ($n < 30$), data were also tested for equality of variance using the Levene's Test. Data that did not meet the assumptions of normality and homogeneity of variances were transformed using the log $(x+1)$ function. Parametric water uptake data were analyzed using a one-way analysis of variance (ANOVA) and non-parametric water uptake data were analyzed using the Kruskal-Wallis test. Post hoc testing of parametric data included Dunnett's Test for the comparison of means of treatment groups to the control group, as well as Tukey's Test to compare the means of treatment groups to one another. Parametric fresh weight data that contained values of zero (indicating no growth) were analyzed using two-sample t-tests

for two independent samples and non-parametric data was analyzed using the Mann-Whitney Test (α =0.05 for all statistical analyses). All statistical analyses were carried out using IBM SPSS Statistics 19 (SPSS Inc. 2010) and all graphs were created using Sigma Plot 10.0 (Systat Software Inc. 2006).

2.3 Results and discussion

2.3.1 Observable phytotoxicity

When compared to the controls, visible phytotoxic effects of tailings treatments were not observed on the leaves, stems, or roots of common reed. However, the phytotoxic effects were clearly visible when examining the cattail grown in MFT reclaim and AFD release water. The phytotoxic effects in cattail included discoloration of leaves, stems, and roots, as well as a visually observable difference in plant root and shoot growth when compared to control cattail (Figure 2.2). These observed decreases in growth compared to controls are supported by the fresh weight data presented in Section 2.3.2 (Figure 2.3).

2.3.2 Fresh weight

Over the 30 day experiment, AFD release water and MFT reclaim water decreased the whole plant fresh weight of cattail (Figure 2.3). No statistically significant differences ($P < 0.05$) were found between the fresh weight of control common reed and common reed grown in oil sands reclaim and release water. However, statistically significant differences ($P < 0.05$) were found between the fresh weight of control cattail when compared to cattail grown in oil sands reclaim and release water. Over the 30 day

experiment, cattail grown in control media had an increase of 97% fresh weight over plants grown in MFT and 100% more than plants grown in AFD Cell 8 and AFD Composite tailings water. Plants that experienced a loss in fresh weight over the course of the experiment (due to desiccation and death) were assigned a value of 0 g for fresh weight.

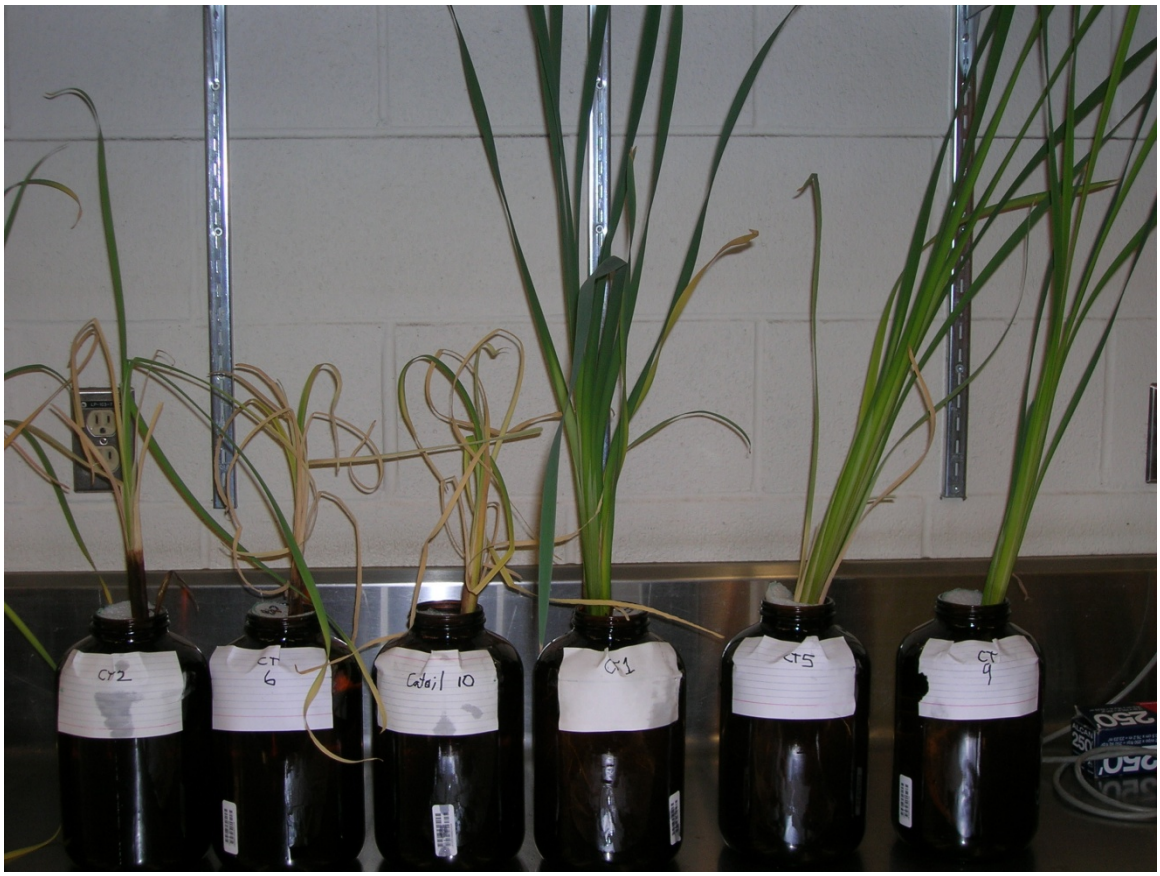

Figure 2.2. Visible phytotoxicity in cattail grown in atmospheric fines drying (AFD) Cell 8 release water (cattail plants labeled CT 2, CT 6, and Cattail 10, on the left hand side of the photograph). The cattail labeled CT 1, CT 5, and CT 9 on the right hand side of the photograph are the control cattail group.

Although the control cattail experienced significantly greater ($P < 0.05$) growth than control common reed, overall common reed grown in oil sands water underwent more growth when compared to its control group. For example, common reed growing in

MFT reclaim water achieved 26% of the control common reed fresh weight, whereas cattail growing in MFT reclaim water only achieved 3% of the control cattail fresh weight. The results found in this experiment are contrasted by those found by Foote and Hornung from a field study whereby cattail grown in oil sands process-affected water (OSPW) had greater above ground biomass increases than plants grown in fresh water and peat soil [Foote & Hornung, 2007]. Foote and Hornung speculated that the observed increase in growth was due to higher nutrient levels in the OSPW.

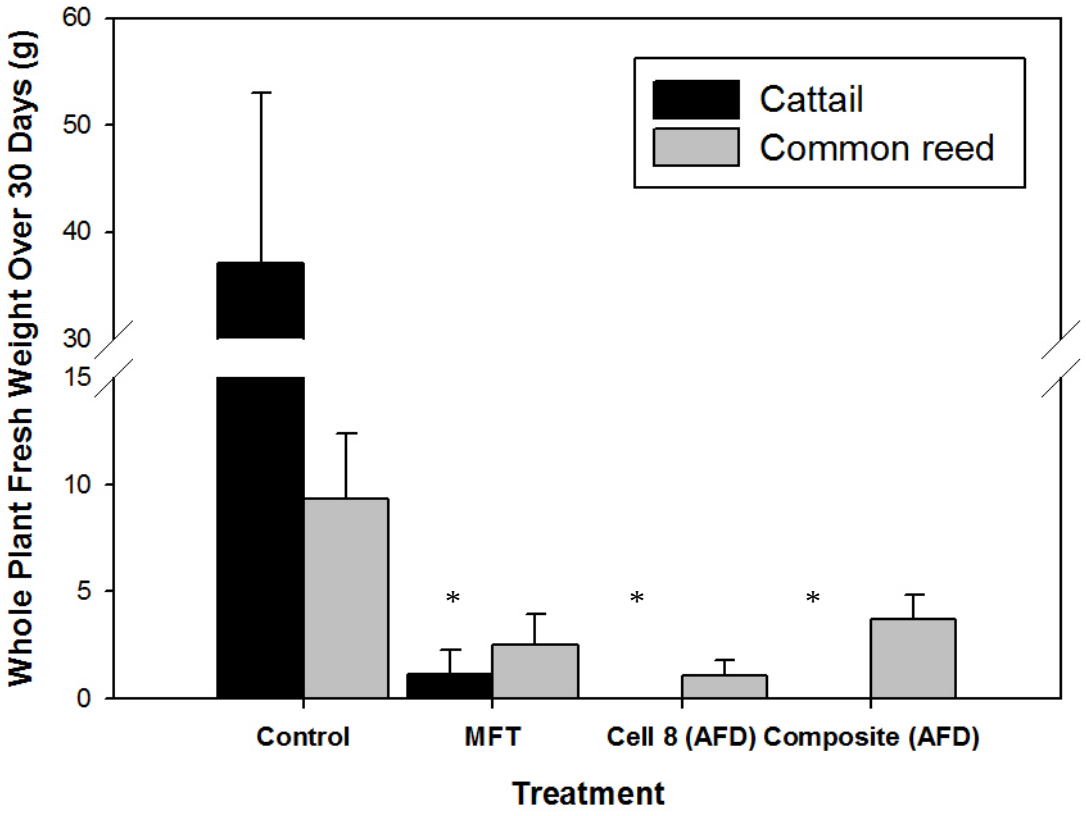

Figure 2.3. Whole plant fresh weight of cattail and common reed over 30 day experiment. Data are the mean (n=3) difference in whole plant fresh weight ± standard error. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between the control group and the group grown in oil sands tailings release water (Cell 8 and Composite) or traditional mature fine tailings (MFT) treatment groups, for each species.

2.3.3 Water uptake

At the conclusion of the study (Day 30), AFD release water and MFT reclaim water had both caused a significant effect ($P < 0.05$) on the water uptake rates of both cattail and common reed (Figures 2.4 and 2.5, respectively). Statistically significant differences ($P < 0.05$) between the water uptake of control and treated common reed were seen as early as Day 5 of the experiment (Figure 2.5), while significant effects ($P < 0.05$) on cattail were not seen until Day 15 (Figure 2.4). This is interesting because when grown in oil sands tailings or oil sands process affected water, common reed is the more tolerant of the two species, so it was expected that the effects on cattail would have been observed earlier in the experiment. This could be an indication of a difference in species sensitivity or adaptability in regards to long-term exposure to oil sands tailings water.

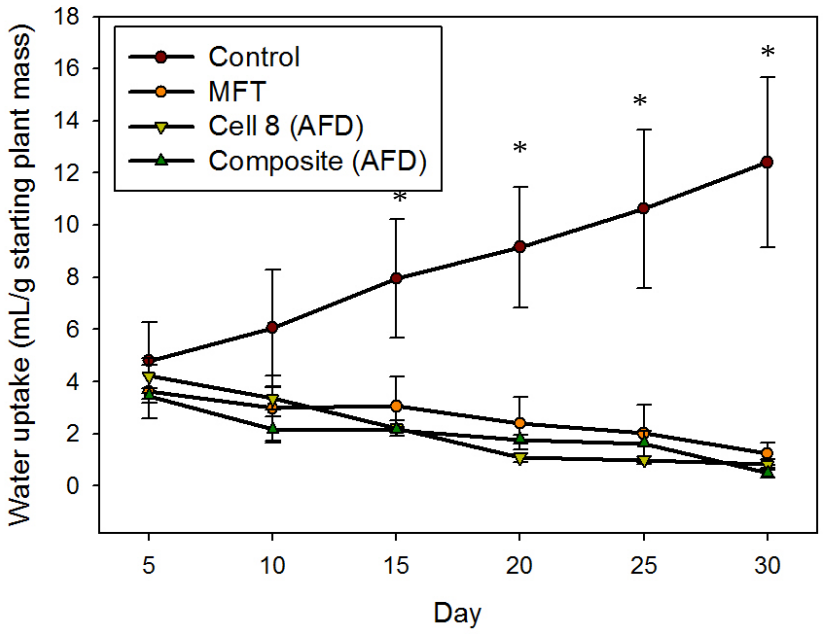

Figure 2.4. Cattail water uptake over 30 day AFD release water experiment. Data are the mean (n=3) volume of water taken up per gram of starting plant mass ± standard error. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between the control cattail and all cattail grown in oil sands tailings release water (Cell 8 and Composite) or mature fine tailings (MFT).

Cattail appears to be more robust to the initial exposure to oil sands tailings water, however over time, common reed is able to adapt to growing in these conditions while it appears that cattail is unable to adapt. Although statistically significant effects ($P < 0.05$) on water uptake were detected, evidence that common reed may be able to adapt to growing in oil sands tailings was observed during the hydroponic experiments. Tailings were not acutely toxic (i.e. no replicates died) and none of the common reed plants stopped taking up water completely. Over the course of the thirty-day experiment, within each treatment, common reed showed significantly ($P < 0.05$) greater water uptake per gram of starting fresh weight than cattail. For example, for the period of Day 26 to Day 30, control common reed had taken up an average of 31.5 mL nutrient media per gram of starting fresh mass, compared to 12.4 mL nutrient media per starting fresh mass in the cattail control group. Moreover, AFD treated common reed plants were also able to continue to produce new shoots and expand their root systems, indicating that they may be able to adapt to growing in AFD tailings water. These findings contrast the research conducted by Armstrong *et al*. [2010] whereby common reed were unable to adapt to growing in simulated runoff water that was produced from dried MFT that had been amended with a polyacrylamide polymer. An explanation for this may be that the AFD release water that was used in this experiment is much more dilute than that tested by Armstrong *et al*. [2010]. The lab scale materials were wetted with much smaller volumes of water than the AFD pilot materials, leading to much more concentrated release water for use in the lab scale phytotoxicity experiments. A different polyacrylamide polymer may have also been used or the naphthenic acids contained within the AFD tailings were from different sources and may not have the same phytotoxicity as the naphthenic acids

in the present study. It is important to note than in the present study, when comparing the effects on water uptake, no significant differences were found between the phytotoxic effects of traditional MFT and the phytotoxic effects of the AFD tailings treatments on both cattail and common reed.

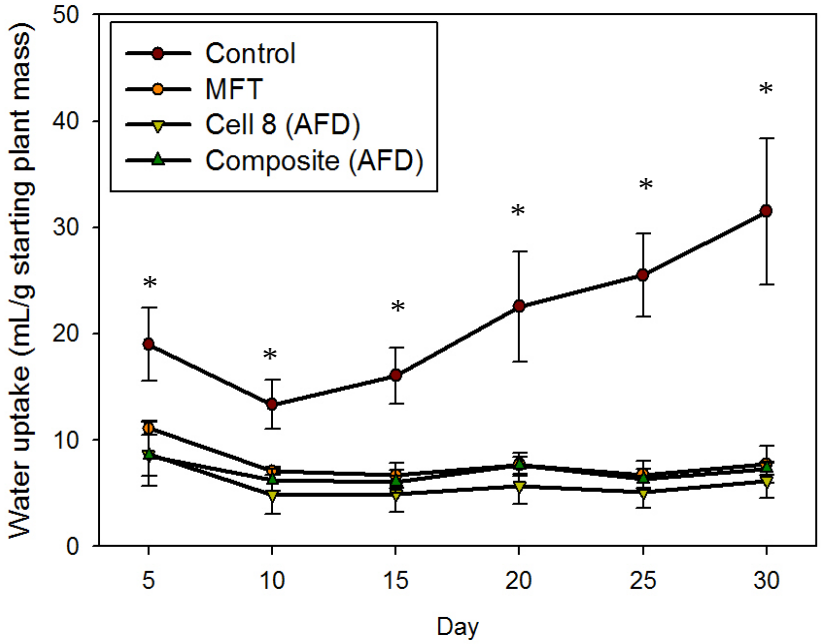

Figure 2.5. Common reed water uptake over 30 day AFD release water experiment. Data are the mean (n=3) volume of water taken up per gram of starting plant mass ± standard error. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between the control common reed and all common reed grown in oil sands tailings release water (Cell 8 and Composite) or mature fine tailings (MFT).

Liquid chromatography-mass spectrometry (LC-MS) analysis of AFD release water determined that acrylamide levels in the water were below the instrument detection limit of 1 µg/L. As expected, it does not appear that the polyacrylamide polymer is being degraded into acrylamide monomers and it is unlikely that the polyacrylamide is contributing to the phytotoxicity of AFD release water. The USEPA does not allow

acrylamide levels in drinking water to exceed 0.5 µg/L, and although this water is not intended for human consumption, because of the potential carcinogenicity of acrylamide, further analysis with more sensitive methods may be warranted.

2.4 Conclusions

The present study revealed that because there are no significant differences between the relative phytotoxicity of MFT reclaim water and AFD release water, AFD processing will not likely increase the phytotoxic effects of oil sands tailings. Common reed appears to be better able to adapt to growing in AFD tailings and is therefore considered to be a more suitable wetland plant species than cattail for use in the long term reclamation of oil sands mining sites.

CHAPTER 3

THE PHYTOTOXICITY OF ATMOSPHERIC FINES DRYING SNOWMELT RUNOFF WATER

3.1 Introduction

The production of one barrel of oil from oil sand requires approximately three barrels of water [Barrow *et al*., 2010] and 75% of this water comes from recycled process water [Long *et al*., 2005]. Due to the regulations on removing water from the Athabasca River, it is necessary for oil sands companies to recycle process water from the extraction process. As a result of the zero discharge policy, oil sands tailings are discharged into large tailings ponds for storage and water recycling. Mature fine tailings (MFT) are formed in tailings ponds when coarse sands settle rapidly, leaving a suspension of residual bitumen and clay fines trapped in the water. Today's current estimate is that there is between 720 - 1000 million m^3 of MFT being stored within the Athabasca Oil Sands Region [Kasperski & Mikula, 2011]. Gravity settling of the fines releases a layer of water that is collected for re-use [Penner & Foght, 2010] and after two to three years of settling, MFT contain approximately 30% wt solids [Wang *et al*., 2010]. It is estimated that the remaining suspended solids contained within process water could take hundreds of years to settle [Johnson & Miyanishi, 2008]. Tailings ponds currently cover an area of approximately 180 km^2 [Kasperski & Mikula, 2011] and are a massive reclamation challenge. Oil sands operators are developing strategies to enhance the efficiency of the settling of the fine suspended solids in order to re-use water released from oil sands tailings and eventually reclaim the land occupied by tailings ponds.

3.1.2 The atmospheric fines drying process

Shell Canada Energy has developed the atmospheric fines drying (AFD) process as part of a long-term tailings management strategy to minimize the volume of slurry oil sands tailings stored in tailings ponds (Section 1.2.1). In short, the AFD process involves adding a polyacrylamide polymer to MFT, and then spreading these flocculated tailings over sloped drying areas (called cells) where the fines are able to settle and water is released and collected in drainage weirs to be recycled back into the extraction process. The purpose of adding the anionic polymer is to flocculate the suspended fines thereby increasing the speed of the fines settling process. As part of the reclamation strategy, the long term intention is to stack these dewatered tailings in a dedicated disposal area (such as a mined out pit) then cap with overburden material.

Investigating the phytotoxicity of the runoff water associated with the AFD process and the long-term stability of the dried tailings deposits is important as these will eventually be incorporated into the reclaimed landscape. Additionally, the phytotoxicity experiments will help determine if cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) are suitable species to be included in future efforts.

3.1.3 Phytotoxic constituents of oil sands tailings water

Naphthenic acids are important to consider when evaluating the phytotoxicity of runoff water from the dried AFD deposits. The phytotoxicity of naphthenic acids to common reed and cattail has been previously described [Armstrong *et al.*, 2008]. Armstrong and co-researchers found that the phytotoxicity induced by naphthenic acids is

manifested by changes in water uptake rates and plant growth and is attributable to altered water relations within the plants. Polyacrylamide phytotoxicity has not been previously reported. However, there is concern regarding the long term stability of polyacrylamide and the potential ground and surface water contamination should the environmental degradation of polyacrylamide occur, forming highly toxic acrylamide monomers [Smith *et al*., 1996; Smith *et al*., 1997].

The goal of this study was to evaluate the phytotoxicity of runoff water collected from dried AFD tailings deposits using two native emergent aquatic macrophytes cattail and common reed. The phytotoxicity of the AFD runoff water was then compared to the phytotoxicity of the AFD release water in order to determine whether there are any changes in the phytotoxicity of the water associated with these dried tailings over time.

3.2 Materials and methods

3.2.1 Hydroponic experiments

Thirty day hydroponic experiments using the emergent macrophytes common reed and cattail were conducted following the methods described by Armstrong *et al*. [2010]. In summary, cattail and common reed root cuttings were mass cultured in a climate controlled growth chamber in quarter-strength modified Hoagland's nutrient medium. After a three-week acclimation period, they were transferred to individual 2.5L glass jars to acclimatize for an additional two weeks. Chamber conditions were maintained at a 16:8 hour, 25ºC:18ºC day/night cycle with an average light intensity of 162 μmol/m^{-2}/s^{-1}. Each plant was supplied with a glass aeration tube attached to an aquarium air pump to supply mixing to the media. A plastic foam plug was fitted into the

opening of the jar, with a slit allowing support to the plant, and a hole to support the glass aeration tube. The plastic foam plug also prevented excess evaporation of the growth medium. Following the two week acclimation period, plants were randomly assigned treatments (Day 0). Control plants were provided with a fresh 2.5 L volume of quarter-strength modified Hogland's nutrient medium (Section 2.2.2). For plants assigned tailings water, the nutrient medium was replaced with 2.5 L of AFD Cell 4, Cell 7, or Cell 8 snowmelt runoff water. Four plants per species were assigned to each treatment type for a total of 32 plants (16 cattail and 16 common reed). Over the 30 day experiment, water uptake was assessed by monitoring the volume of water taken up by the plants and jars were topped up to the original 2.5 L volume every five days (on Days 5, 10, 15, 20, 25, and 30) with tailings runoff water or control nutrient media. Prior to each use, tailings runoff water was stirred for approximately one minute to achieve a uniform mixture. In order to minimize the variability in water uptake data among replicates, water uptake data was normalized to starting fresh weight and is therefore reported as milliliter of water per gram starting fresh weight. Fresh weight was monitored by recording the mass of each plant on Day 0 and then again on Day 30.

3.2.2 Data analysis

All data were tested for normality using the Shapiro-Wilk Test ($n < 30$), data were also tested for equality of variance using the Levene's Test. Data that did not meet the assumptions of normality and homogeneity of variances were transformed using the log $(x+1)$ function. Parametric water uptake data were analyzed using a one-way analysis of variance (ANOVA) and non-parametric water uptake data were analyzed using the

Kruskal-Wallis test. Post hoc testing of parametric data included Dunnett's Test for the comparison of the means of treatment groups to the control group, as well as Tukey's Test to compare the means of treatment groups to one another. Parametric fresh weight data that contained values of zero (indicating no growth) were analyzed using two-sample t-tests for two independent samples and non-parametric data was analyzed using the Mann-Whitney Test (α =0.05 for all statistical analyses). Tailings water chemistry data were analyzed using linear regression. All statistical analyses were carried out using IBM SPSS Statistics 19 (SPSS Inc. 2010) and all graphs were created using Sigma Plot 10.0 (Systat Software Inc. 2006).

3.3 Results and discussion

3.3.1 Observable phytotoxicity

At the conclusion of the 30 day experiment, no visible signs of phytotoxicity (discoloration, desiccation/curling of leaves, and reduced growth) were observed on the common reed plants grown in AFD runoff water. Some visible desiccation and discoloration of the leaves of cattail was observed (Figure 3.1), however the severity was much less than what was observed in the previous experiment evaluating AFD release water (Chapter 2). A decrease in observable phytotoxicity was expected because the tailings runoff water being evaluated was more dilute than the release water examined in Chapter 2 (See Table 3.1 for water chemistry).

Figure 3.1. Visible phytotoxicity in cattail grown in atmospheric fines drying (AFD) Cell 8 runoff water (four cattail plants labeled Cell 8, on the left hand side of the photograph). The four cattail right hand side of the photograph are the control cattail group.

3.3.2 Fresh weight

The common reed whole plant fresh weight data collected during this experiment showed some unexpected results (Figure 3.2). The common reed plants grown in AFD snowmelt runoff water showed significantly ($P < 0.05$) greater fresh weight than the control common reed plants. Over the 30 day experiment, the control common reed group had an increase in fresh weight of 6.4 g while the common reed plants grown in AFD runoff water had a fresh weight increase of 34.2 g to 39.5 g. When compared to the size of the control plants, there were no observable differences in the size of the plant shoot of the common reed plants grown in AFD runoff water. However, the roots of the

common reed grown in AFD snowmelt water were enlarged compared to controls (Figure 3.3). A constituent or mixture of constituents in the snowmelt runoff water likely stimulated the growth of the roots of common reed, however it was not determined if this is a phytotoxic response. The roots of the common reed plants grown in the AFD runoff water had visible increases in length and diameter compared to those of common reed grown in control nutrient media (data not reported).

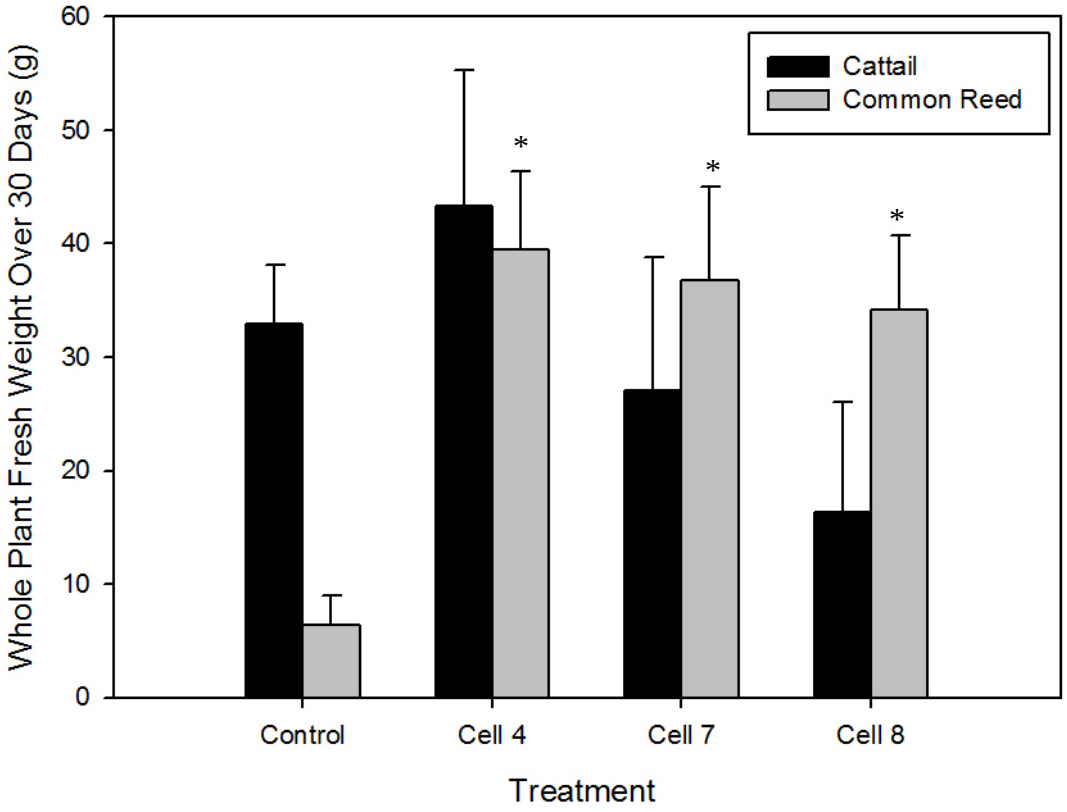

Figure 3.2. Whole plant fresh weight of cattail and common reed over 30 day atmospheric fines drying (AFD) snowmelt runoff water experiment. Data are the mean (n=4) difference in whole plant fresh weight ± standard error. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between the control group and the group grown in the AFD deposit snowmelt runoff water.

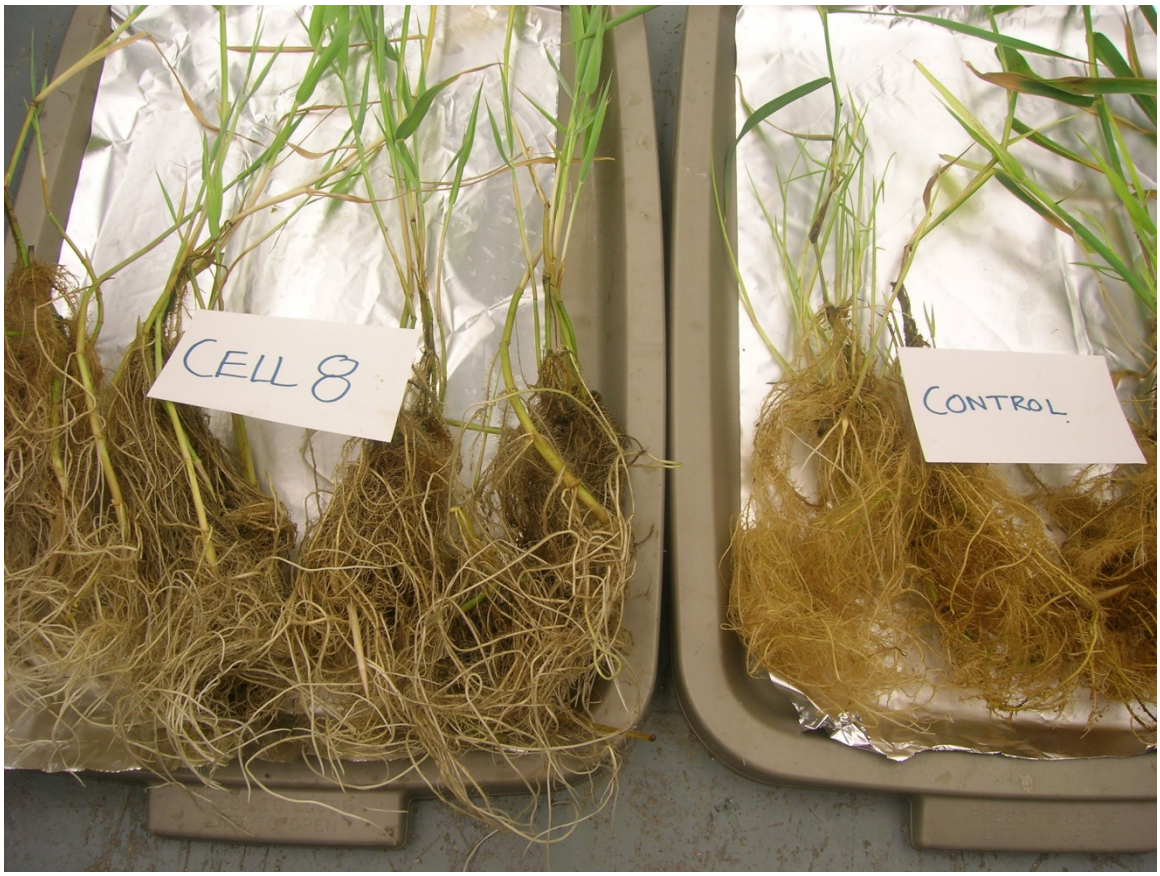

Figure 3.3. The increased growth of the roots of common reed grown in atmospheric fines drying (AFD) snowmelt runoff water can be seen in the common reed plants in the left hand side of the photograph (labeled Cell 8). The common reed plants on the right hand side of the photograph are the control group.

The same trend was not seen in cattail - no significant ($P < 0.05$) differences were found between the whole plant fresh weight of control cattail and cattail grown in AFD runoff water (Figure 3.2).

3.3.3 Water Uptake

The water uptake of AFD runoff water in cattail and common reed are reported in Figure 3.4, and Figure 3.5, respectively. Cattail grown in the AFD snowmelt runoff water showed significantly ($P < 0.05$) decreased water uptake by Day 15 of the experiment

(Figure 3.4). Over the period of Day 26 to Day 30, control cattail took up 5.4 mL of nutrient media per gram of starting fresh weight while cattail growing in AFD Cell 8 runoff water took up 2.0 mL runoff water per gram of starting fresh weight.

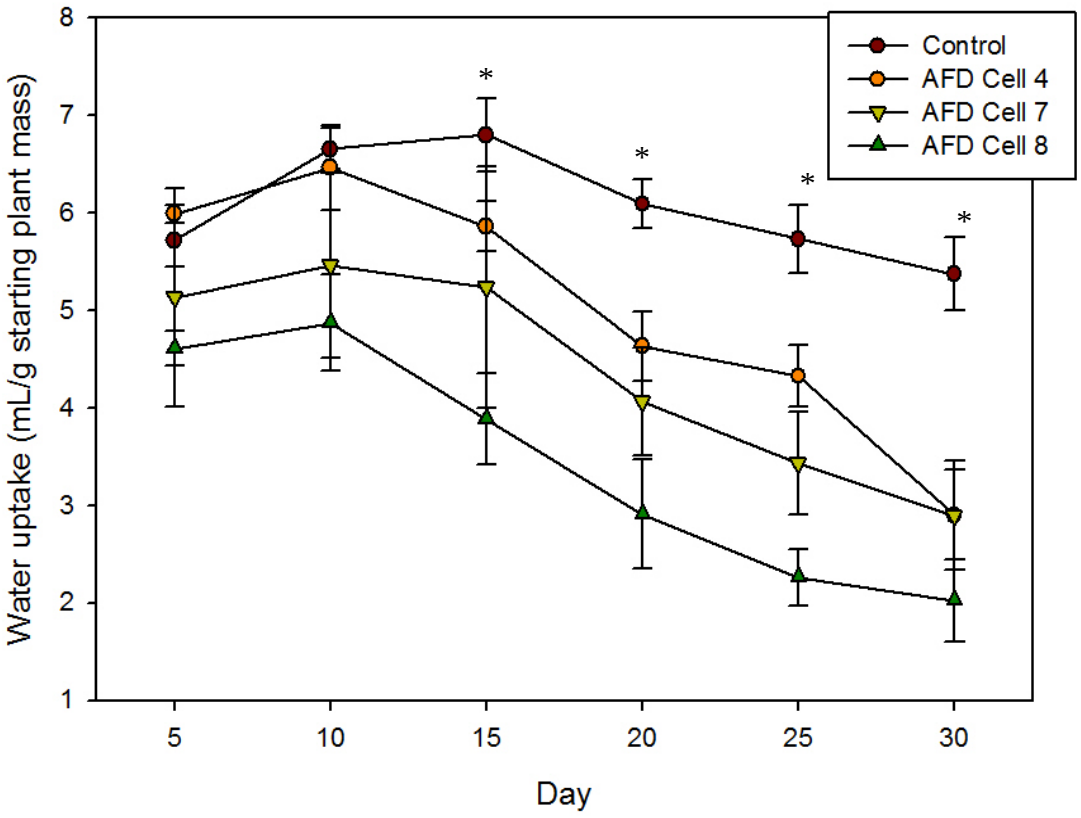

Figure 3.4. Cattail water uptake over 30 day AFD snowmelt runoff experiment. Data are the mean (n=4) volume of water taken up per gram of starting plant mass ± standard error. An asterisk (*) indicates a statistically significant difference (P <0.05) between the control cattail and all cattail grown in snowmelt runoff water collected from the AFD tailings deposit.

The water uptake data for common reed follows the same trend as the fresh weight data represented in Figure 3.2. In general, common reed grown in AFD tailings runoff water took up more water than common reed grown in control nutrient media. Statistically significant differences (P < 0.05) between the water uptake of control common reed and common reed grown in AFD runoff water were observed starting on

Day 15 of the experiment. In the final five days of the experiment (Day 26 to Day 30), control common reed took up 7.4 mL of nutrient media per gram of starting fresh weight while common reed grown in AFD runoff water took up between 12.7 mL and 13.8 mL of water per gram of starting fresh weight (Figure 3.5). Over the course of the 30 day experiment, the control common reed plants took up 8.8 mL nutrient media per gram of starting fresh weight while common reed grown in AFD runoff water took up between 12.2 and 12.7 mL runoff water per gram of starting fresh weight. These values are expected due to the observed increase in fresh weight of common reed grown in AFD runoff water. However, further investigation is required to evaluate if there is an additional constituent in the runoff water that induced the root growth in common reed. It is speculated that the rain or snow that fell on the AFD deposit contained additional or a greater concentration of nutrients than that present in the control growth media (See Section 3.3.4 for water chemistry data, also refer to Appendix A for raw data). It is interesting to note that this abnormal growth seems to be species specific, as it was not observed in the cattail grown in the same tailings materials under the same growing conditions.

Stimulatory effects of naphthenic acids on plants have been reported. Wort *et al.* [1973] conducted a study where fourteen-day-old bush bean (*Phaseolus vulgaris*) plants were sprayed with potassium naphthenate at a concentration of 20mM. Thirty days after the application of potassium naphthenate, plants were found to have an increase in the number and weight of green pods, and an overall increase in dry mass when compared to control plants [Wort *et al*., 1973]. Wort *et al*. also found levels of increased of activity in the enzymes involved in nitrogen metabolism. Increased rates of photosynthesis were

found in cattail growing in wetlands receiving oil sands effluent [Bendell-Young *et al*., 2000]. These increased rates of photosynthesis were not however accompanied by an increase in plant growth.

Lab scale hydroponic experiments using common reed have previously been conducted examining the phytotoxic effects of different MFT drying chemical amendments and simulated runoff water from dried tailings [Armstrong *et al*., 2010]. Within the study, the phytotoxic effects of simulated runoff water from dried MFT amended with a polyacrylamide polymer were evaluated. Simulated runoff water from the polymer treated dried tailings was found to be acutely toxic to common reed, with the plants dying shortly after the beginning of the experiment (water uptake ceased by Day 5 of the experiment). The differences in phytotoxicity between the natural runoff water in this study and the simulated runoff water in the Armstrong study are likely due to the dilution of the tailings water being tested which is a product of the different scales of the two experiments. The runoff water collected for use in this experiment was collected from a pilot scale AFD operation and is therefore a more realistic representation of how AFD runoff water may affect the surrounding landscape with respect to the aquatic macrophytes present.

In the present study, no statistically significant ($P < 0.05$) differences were found between the water uptake data (both species) for the individual AFD cells. In order to compare the phytotoxicity of AFD release and runoff waters, the water uptake data for the two experiments were plotted on the same graph (cattail Figure 3.6; common reed Figure 3.7). It was observed that overall, throughout the 30 day experiments, cattail grown in runoff water from the AFD deposits took up more water per gram of starting

fresh weight than cattail grown in MFT reclaim and AFD release water. The water uptake data for common reed followed the same trend. These observations are expected because the AFD snow melt runoff water was anticipated to be more dilute than the original release water, therefore it was expected that AFD runoff water would be less phytotoxic to cattail and common reed. Another possible explanation could be from the degradation of naphthenic acids (a phytotoxic constituent of oil sands tailings). It has been reported that naphthenic acids have the potential to be biodegraded by microorganisms [Kannel & Gan, 2012].

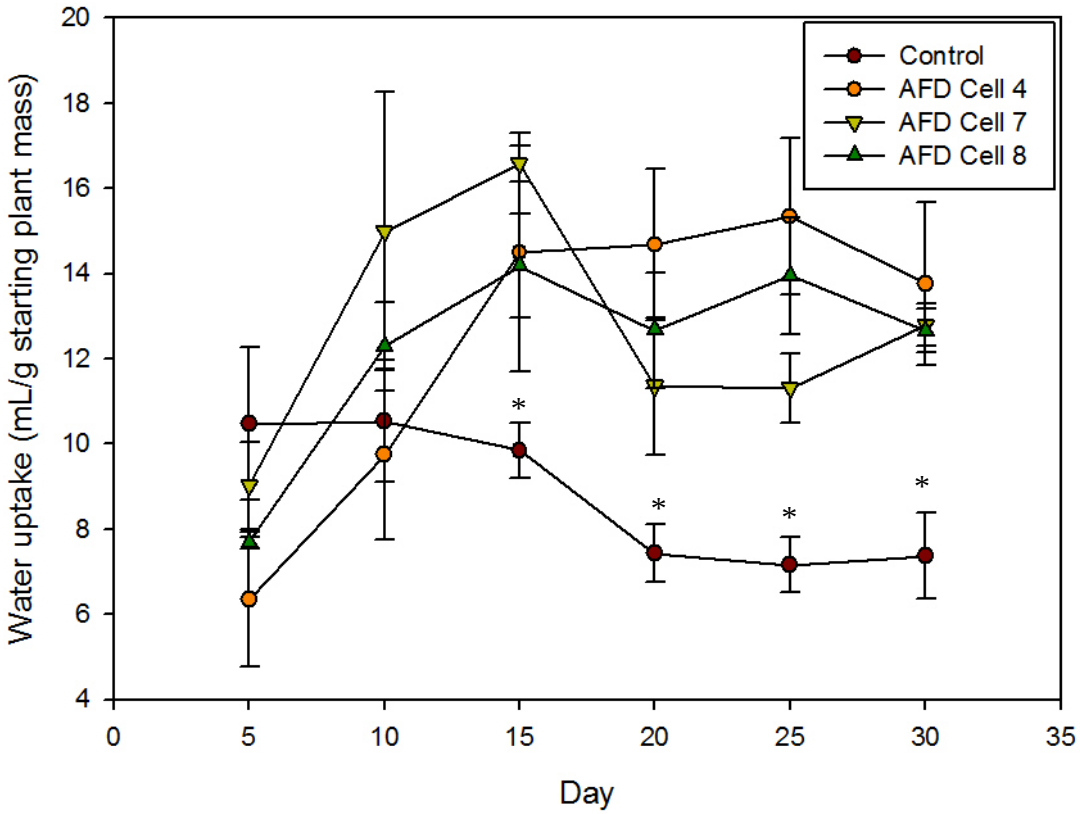

Figure 3.5. Common reed water uptake over 30 day snowmelt runoff experiment. Data are the mean (n=4) volume of water taken up per gram of starting plant mass ± standard error. An asterisk (*) indicates a statistically significant difference (P <0.05) between the control common reed and all common reed grown in snowmelt runoff water collected from the AFD tailings deposit.

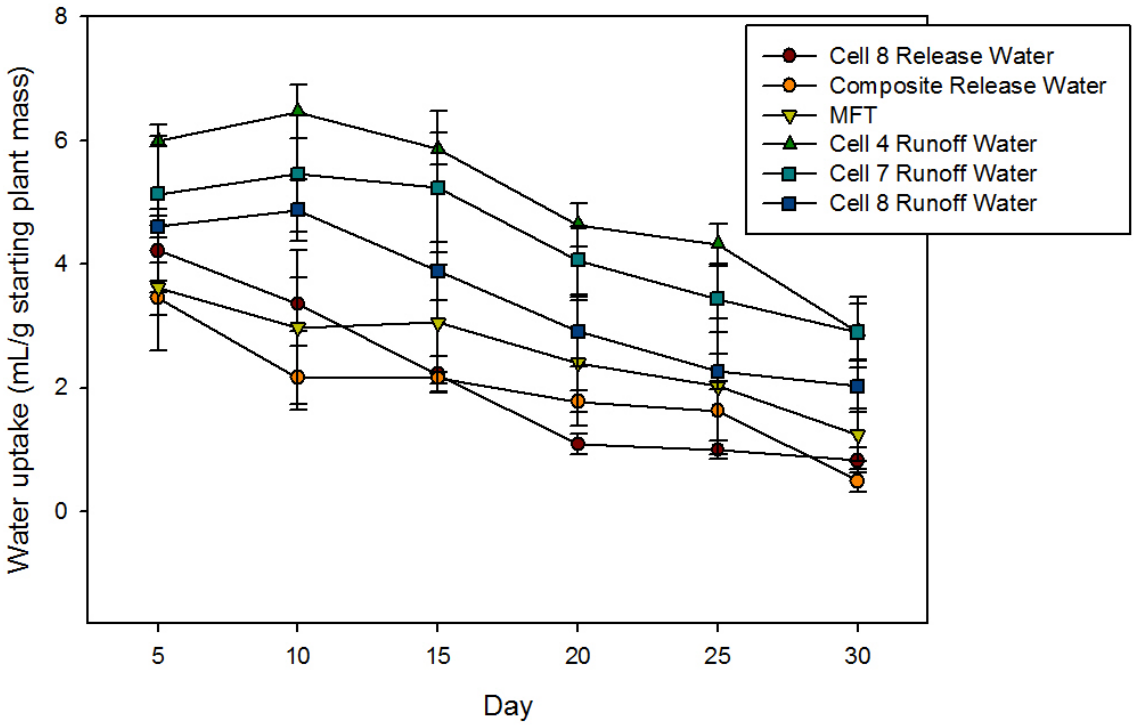

Figure 3.6. Cattail water uptake over 30 day atmospheric fines drying (AFD) release water experiment (n=3) and 30 day AFD snowmelt runoff water experiment (n=4). Data are the mean volume of water taken up per gram of starting plant mass ± standard error.

The runoff water collected in the spring for the phytotoxicity experiments had percolated through AFD tailings deposits that had been deposited the previous fall. Microorganisms present may have degraded the naphthenic acids into less phytotoxic compounds, which could be a factor in the decrease in phytotoxicity between the release water collected in the fall when the tailings were deposited, and runoff water collected the following spring. Lastly, it is possible that the snowmelt runoff water collected did not fully mix with the frozen tailings deposit.

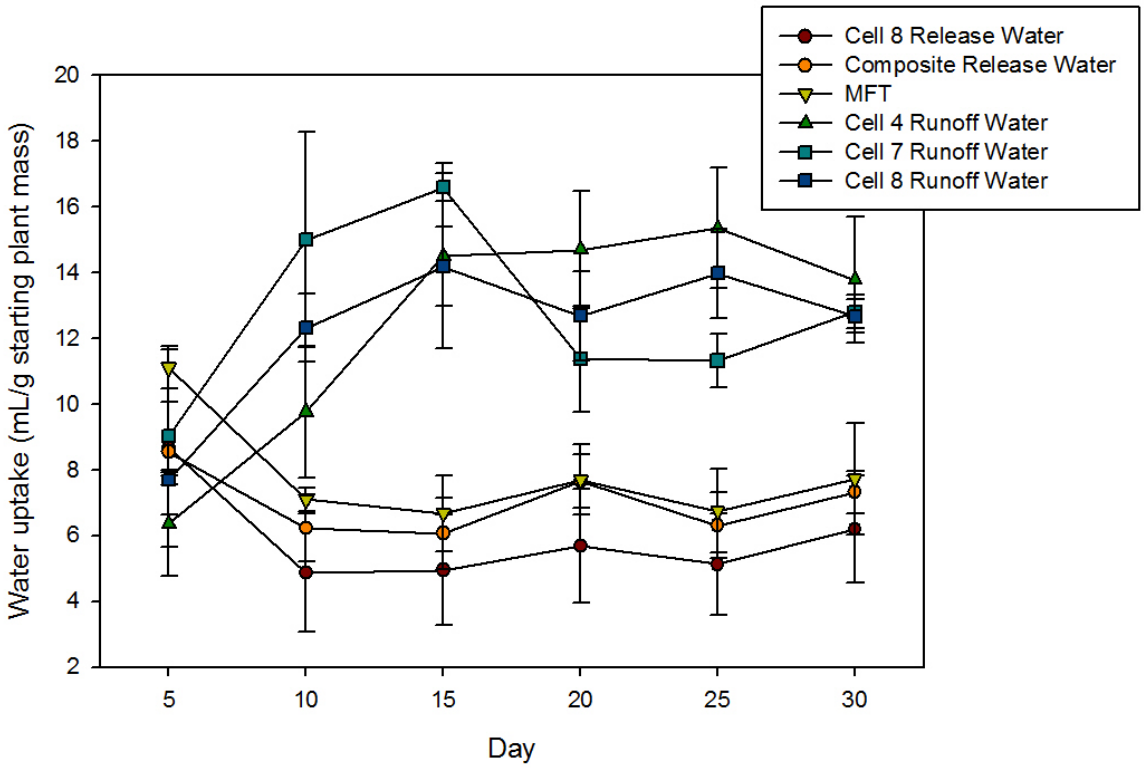

Figure 3.7. Common reed water uptake over 30 day atmospheric fines drying (AFD) release water experiment (n=3) and 30 day AFD runoff water experiment (n=4). Data are the mean volume of water taken up per gram of starting plant mass ± standard error.

3.3.4 Water chemistry

Water chemistry was analyzed by the Saskatchewan Research Council (SRC) for oil sands tailings water samples (Table 3.1; Appendix A for raw data). Major ion analysis was conducted using inorganic chemistry and inductively coupled plasma mass spectrometry (ICP-MS). Linear regression was used to determine if a relationships exist between the fresh weight of the plants over the 30 day experiment and the concentration of various ions in MFT reclaim water, AFD release water, and AFD runoff water. Given the concentrations of ions detected in the samples, no significant relationships were detected between the whole plant fresh weight of either species over the 30 day experimental period and any of the parameters listed in Table 3.1. Furthermore, when

compared to the quarter-strength Hoagland's nutrient media, it does not appear that the AFD runoff water contains greater concentrations of nutrients that may be responsible for the increased growth. For example, the control media contains 53 mg/L nitrogen, 59 mg/L potassium, 55 mg/L calcium, and 12 mg/L magnesium [Hoagland & Arnon, 1950].

Table 3.1. Water chemistry data for Shell Canada's Muskeg River Mine oil sands tailings water. Mature fine tailings (MFT) release water and atmospheric fines drying (AFD) composite and Cell 8 release water samples were collected in the fall of 2010. AFD snowmelt runoff water samples (Cell 4, Cell 7, and Cell 8 runoff) were collected in the spring of 2011. A value of '0' indicates that the ion was not detected at a level greater than 1 mg/L. Data are milligrams of ion per liter of water (n=1) (Appendix A).

Sample ID	HCO_3^-	CO_3^-	Cl^-	OH^-	NO_3^-	Ca^{2+}	Mg^{2+}	K^+	Na^+	SO_4^{2-}	pH
Shell MFT reclaim	460	10	220	0	0.75	23	12	18	314	180	8.44
Composite release	603	11	285	0	15	31	13	15	362	100	8.43
Cell 8 release	570	29	292	0	13	26	13	15	369	95	8.61
Cell 4 runoff	439	4	178	0	0.56	43	16	10	227	110	8.38
Cell 7 runoff	289	4	148	0	1.2	30	9.0	6.9	209	160	8.39
Cell 8 runoff	556	5	311	0	1.1	32	12	12	373	120	8.48

Previous studies examining snowmelt water collection indicate that the first 30% of snowmelt water contains 50-80% of the pollutant load [Johannessen & Henriksen, 1978]. Studies have found that lower melt rates result in higher contaminant concentrations [Colbeck, 1981] therefore depending on the intensity of the increase in spring temperatures and the length of the spring melt the samples collected from the AFD deposit in the present study may have had drastically different chemical concentrations. In a study conducted by Tatarniuk *et al*. [2009], it was found that pollutant and ion concentrations in melt water decreased significantly as the melting season progressed. The samples collected for use in this experiment were collected near the end of the spring runoff, where most if not all of the snow on each cell had melted. In general, the ion concentrations were lower in the AFD runoff samples compared to the AFD release water

samples (Table 3.1). AFD release water had consistently elevated concentrations of potassium, sodium, and chloride ions which could be an indication to why the cattail and common reed grown in these tailings waters had lower water uptake rates. The present study gives a preliminary indication of the ability of cattail and common reed to grow in runoff water from a dry AFD deposit. In order to determine a more comprehensive representation of the runoff water coming from the AFD deposits, it would be appropriate to conduct a study evaluating the phytotoxicity of water samples collected throughout the spring runoff.

3.4 Conclusions

The phytotoxicity experiments conducted using AFD runoff water demonstrate that the aquatic macrophytes common reed and cattail are able to grow in snowmelt runoff water coming from dried AFD tailings deposits. The present study also indicates that the runoff water from the AFD deposits is less phytotoxic to cattail and common reed than the initial release water which may be an indication of that these two aquatic species can be incorporated into future reclamation strategies. Common reed is more tolerant to growing in AFD runoff water and is therefore considered to be the more suitable of the two wetland plant species to be included in the long term oil sands reclamation strategies.

CHAPTER 4

THE PHYTOTOXICITY OF CONSOLIDATED TAILINGS RELEASE WATER AND NUTRIENT MEDIA SPIKED WITH NAPHTHENIC ACIDS

4.1 Introduction

Mature fine tailings (MFT) are a stable suspension of fine solids that form in tailings ponds [Demoz & Mikula, 2012] following a period of three to five years once fluid tailings have reached approximately 30 % wt solids [Kasperski & Mikula, 2011]. Further settling of the suspended solids contained within MFT is very slow and over time, these tailings have been accumulating in tailings ponds which currently cover over 180 km^2 of the Athabasca oil sands region in Canada (Figure 1.1) [Kasperski & Mikula, 2011]. Approximately 1.4 barrels of non-settling MFT results from each barrel of ore that is mined. These 'soft' tailings are a major hurdle in the reclamation process of oil sands tailings ponds because these materials cannot be load bearing [Demoz & Mikula, 2012]. Aged MFT is composed of over 80% water and this water cannot be released for recycling without further chemical amendments [Demoz & Mikula, 2012]. In order to minimize the volume of water needed for oil sands processing, oil sands operators have been developing technologies to enhance the efficiency of the MFT settling process. Enhancing the settling of MFT will permit process water trapped within MFT to be recycled back into the extraction process. Shell Canada has developed the atmospheric fines drying (AFD) process whereby a polyacrylamide polymer is added to MFT and these flocculated tailings are then thinly spread over a large sloped area and allowed to rapidly dewater (Section 1.2.1). Water released from these tailings is collected and returned to the extraction process.

An older method developed for speeding up the settling of MFT is called the consolidated or composite tailings (CT) process. The CT process is being evaluated at Natural Resources Canada's CanmetENERGY Research Center (Canmet) in Devon, Alberta, Canada. The CT process involves the addition of sand and gypsum to tailings formed during the extraction process. Gypsum (calcium sulphate; $CaSO_4$) acts as a coagulant, enabling the sand and fines to settle together. The mixture is then transported to a tailings pond, and following a period of approximately one year, a deposit consisting of 70-80 % wt solids remains. The release water is then pumped away to be recycled and a dry deposit is left behind [Kasperski & Mikula, 2011].

Previous research has evaluated the suitability of several plant species as part of the reclamation strategies involving 'soft' tailings [Renault *et al*., 1998; 2003; 2004; Redfield *et al*., 2003; Armstrong *et al*., 2010]. Due to the increased salinity of oil sands tailings, remediation studies evaluating the phytotoxicity of CT materials have tended to focus on saline-tolerant species and the majority of these have been terrestrial species [Renault *et al*., 1998; 2003; 2004; Redfield *et al*., 2003]. CT release water has been incorporated into several phytotoxicity studies evaluating the germination, survival, and growth of raspberry, strawberry, willow, aspen, dogwood, white and black spruce, lodgepole pine, slender wheatgrass, barley, and altai wildrye [Renault *et al*., 1998; 2003; 2004]. Redfield *et al*., [2003] studied the ability of red-osier dogwood seedlings to grow in CT release water. A study assessing the potential use of the aquatic macrophyte common reed as part of the reclamation strategy has also been conducted [Armstrong *et al*., 2010].

The objective of this study was to evaluate the phytotoxicity of CT release water, with or without gypsum added, to the aquatic macrophytes cattail and common reed. In order to evaluate if any phytotoxicity observed in the CT release water experiment was caused by a combination of the constituents of the CT release water (high salinity, gypsum, high pH, naphthenic acids) or solely by naphthenic acids (Chapter 5), a naphthenic acid dosing experiment was also conducted that evaluated the phytotoxicity of the naphthenic acids, without the presence of other potentially phytotoxic constituents.

4.2 Materials and methods

4.2.1 Extraction of naphthenic acids from mature fine tailings (MFT)

For the naphthenic acid dosing study, quarter-strength modified Hoagland's nutrient solution was spiked with naphthenic acids that had been extracted from Shell MFT. A liquid-liquid extraction method was used to extract naphthenic acids from 200 L of Shell MFT to make a concentrated extract with a final volume of approximately 1 L and a naphthenic acid concentration of 3888 mg/L. The naphthenic acid extraction method used is a modified version of that described by Rogers *et al*. [2002], Janfada *et al*. [2006], and used by Armstrong *et al*. [2008]. In summary, 1 L of MFT (pH adjusted to 2.5 using 18.76 M H_2SO_4) was mixed with 0.5 L of dichloromethane in a 2 L separatory funnel and was agitated with venting for approximately three minutes. The contents were then left to settle for approximately three minutes until separate aqueous and solvent phases developed. The solvent phase was collected in a round bottom flask and evaporated on a rotary evaporator with vacuum (45°C, 630 psi) until a final volume of approximately 15 mL remained in the flask. Once the concentrated extract had been collected from 20 L of MFT, the extract was allowed to evaporate (at room temperature)

to completeness. The extract was then reconstituted in 150 mL of 0.1 M NaOH and was then filtered using a stirred-cell ultrafiltration system. The final extract had a concentration of 3888 mg/L naphthenic acids and was stored in the dark at 4°C.

4.2.2 Consolidated tailings release water

Sixty liters of each type of CT release water (with or without gypsum) was collected and provided by Canmet. Release water was stored at 4°C in 20 L non-leaching plastic pails until used in the experiment. Before use in the hydroponic experiments, release water was stirred for approximately one minute until a homogenous mixture was obtained.

4.2.3 Hydroponic experiments

A thirty day hydroponic experiment using the emergent macrophytes common reed and cattail were conducted following the methods described by Armstrong *et al*. [2010]. Cattail and common reed root cuttings were mass cultured in a climate controlled growth chamber in quarter-strength modified Hoagland's nutrient medium (Section 2.2.2) for at least three weeks before being transferred to individual 2.5 L glass jars to acclimatize for an additional two weeks. Chamber conditions were maintained at a 16:8 hour, 25ºC:18ºC day/night cycle. Each plant was supplied with a glass aeration tube attached to an aquarium air pump to supply mixing to the contents. A plastic foam plug was fitted into the opening of the jar, with a slit allowing support to the plant, and a hole to support the glass aeration tube. The plastic foam plug also prevented excess evaporation of the growth medium. Following the two week acclimation period, plants

were randomly assigned treatments (Day 0). Control plants were provided with a fresh 2.5 L volume of quarter-strength modified Hoagland's nutrient medium. Plants designated for the naphthenic acid dosing study were provided with 2.5 L of fresh nutrient media, which was spiked with 25.5 mL of naphthenic acid extract to achieve a naphthenic acid concentration of approximately 40 mg/L. This concentration was chosen because it is the average naphthenic acid concentration measured from the atmospheric fines drying (AFD) release water samples supplied by Shell Canada (data not shown; see Section 1.2.1 for a description of the AFD process). For plants being evaluated for the phytotoxicity of CT release water, the nutrient media was replaced with 2.5 L of CT release water. Two separate CT release waters were evaluated: 1) water that was released from CT that had been treated with gypsum, and 2) water that was released from CT that did not have gypsum added. Four plants per species were assigned to each treatment for a total of 32 plants (16 cattail and 16 common reed). Overall plant health was assessed by measuring the whole plant fresh weight and water uptake of each individual plant over the thirty day exposure period. Water uptake was assessed by monitoring the volume of water taken up by the plants by topping the jars up to the original 2.5 L hydroponic volume every five days on Days 5, 10, 15, 20, 25, and 30. The jars containing control plants and naphthenic acid dosing plants were topped up with fresh nutrient media, and the jars containing CT release water plants were topped up with their specific CT treatment (CT release water, with or without gypsum). In order to minimize the variability in water uptake data among replicates, water uptake data was normalized to starting fresh weight and is therefore reported as milliliter water per gram starting fresh

weight. Whole plant fresh weight was monitored by recording the mass of each plant on Day 0 and then again on Day 30 of the experiment.

4.2.4 Data analysis

All data were tested for normality using the Shapiro-Wilk Test ($n < 30$), and also tested for equality of variance using the Levene's Test. Data that did not meet the assumptions of normality and homogeneity of variances were transformed using the log (x+1) function. Parametric water uptake data were analyzed using a one-way analysis of variance (ANOVA) and non-parametric water uptake data were analyzed using the Kruskal-Wallis test. Post hoc testing of parametric data included Dunnett's Test for the comparison of the means of treatment groups to the control group, as well as Tukey's Test to compare the means of treatment groups to one another. Parametric fresh weight data that contained values of zero (indicating no growth) were analyzed using two-sample t-tests for two independent samples and non-parametric data was analyzed using the Mann-Whitney Test ($\alpha = 0.05$ for all statistical analyses). Tailings water chemistry data were analyzed using linear regression. All statistical analyses were carried out using IBM SPSS Statistics 19 (SPSS Inc. 2010) and all graphs were created using Sigma Plot 10.0 (Systat Software Inc. 2006).

4.3 Results and discussion

4.3.1 Observable phytotoxicity

When compared to the control plants, subtle signs of phytotoxicity (minor discoloration of leaves, some leaf drying) were observed in common reed grown in CT

release water from tailings with and without gypsum added, as well as nutrient media spiked with naphthenic acids. Interestingly, the roots of common reed grown in CT release water without gypsum were very dense and individual roots were thicker and longer than those of the plants grown in control nutrient media. Visible signs of phytotoxicity (desiccated leaves, discoloration of leaves and roots) were also observed in the cattail grown in CT release water from tailings with and without the gypsum amendment, as well as naphthenic acid spiked nutrient media. The observed phytotoxic effects were much more severe in cattail grown in CT release water from tailings with gypsum added, and visible effects were the least severe in the cattail grown in the naphthenic acids spiked nutrient media (Figure 4.1).

In the present experiment, no mortality was observed in either species for any of the treatment groups, which indicates a lack of acute phytotoxicity. It was previously found that release water from CT tailings with gypsum added caused a loss of leaves in willow and aspen seedlings [Renault *et al*., 1998]. Redfield *et al*. [2003] found that red-osier dogwood seedlings grown in CT tailings substrate and watered with Hoagland's nutrient media made with CT release water containing gypsum had decreased rates of survival. The CT tailings substrate was replaced with reclamation soil, and the seedlings were again watered with Hoagland's nutrient media that had been made with CT release water containing gypsum. The plants were then able to grow and no significant decreases ($P < 0.001$) in dry weight or shoot length were observed [Redfield *et al*., 2003]. The nutrients available to cattail and common reed in the CT release water hydroponic experiment were contained solely within the CT release water. It would be interesting to

evaluate the growth of cattail and common reed in CT release water that has been diluted with nutrient media.

Figure 4.1. The four cattail grown in quarter-strength modified Hoagland's nutrient media spiked with naphthenic acids (40 mg/L) are shown on the left hand side of the photograph (labeled 'AFD Dose'). The four cattail on the right hand side represent the control cattail that were grown in quarter-strength Hoagland's nutrient medium.

4.3.2 Fresh weight

Release water from CT treated with gypsum and nutrient media spiked with naphthenic acids significantly reduced the whole plant fresh weight of cattail ($P < 0.05$; Figure 4.2). Overall, control cattail growing in nutrient media had an increase in fresh weight of 32.9 g over the 30 day experiment while cattail growing in release water from CT treated with gypsum had an increase of 8.7 g. There was no significant difference ($P > 0.05$) found between the fresh weight of cattail grown in CT without gypsum added and control cattail (cattail grown in CT without the gypsum amendment had an increase in fresh weight of 37.9 g over the 30 day experiment). This indicates that the phytotoxicity observed in cattail grown in CT tailings with gypsum may be attributable to the gypsum and not the tailings themselves or that the phytotoxicity observed was simply the additive effects of the tailings and the gypsum. It should be noted that there was a significant difference ($P < 0.05$) found between the fresh weight of cattail grown in CT release water with gypsum and CT release water without gypsum, which may indicate possible species sensitivity to gypsum.

Nutrient media spiked with naphthenic acids also caused a significant reduction ($P < 0.05$) in the whole plant fresh weight of cattail and exposed cattail had an increase in fresh weight of 10.8 g over the 30 day experiment. It was previously found that both 30 mg/L and 60 mg/L doses of non-ionized naphthenic acids (media pH 5.0) caused a significant reduction in the fresh weight of hydroponically grown cattail however, the same doses of ionized naphthenic acids (media pH 7.8) did not significantly decrease fresh weight [Armstrong *et al.*, 2009]. The pH of the nutrient media used in the present

study was between pH 7 and 8, indicating that the majority of the naphthenic acids present were in the less phytotoxic ionized form.

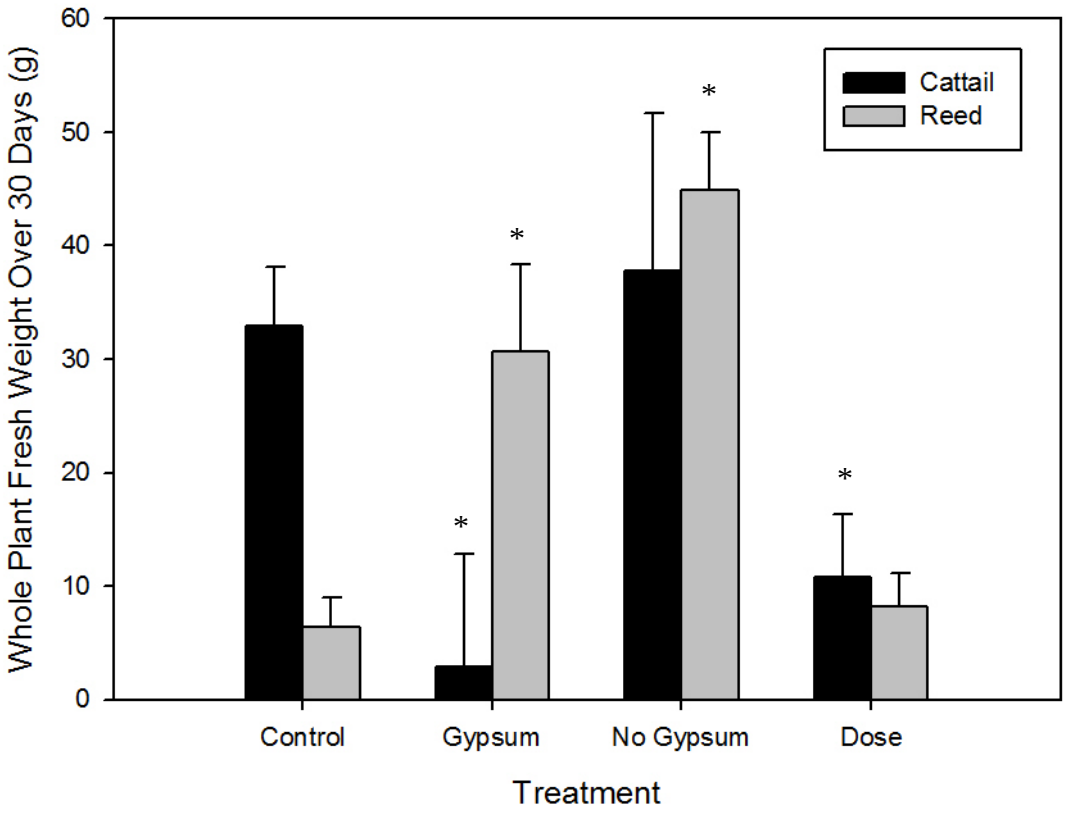

Figure 4.2. Whole plant fresh weight of cattail and common reed over the 30 day experiment evaluating the phytotoxicity of consolidated tailings and 40 mg/L naphthenic acids. Data are the mean (n=4) difference in whole plant fresh weight ± standard error. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between the control and treatment groups.

Both types of CT release water caused a significant change ($P < 0.05$) in the whole plant fresh weight of common reed (Figure 4.2). Unexpectedly, an increase in fresh weight was observed for the common reed grown in CT release water (with and without gypsum). As previously indicated in Section 3.3, there was a considerable increase in the length and thickness of the roots of common reed grown in release water.

Manifestations of rhizotoxicity can include abnormal branching, thickening, and stunting of roots [Michaud *et al*., 2008]. For example, previous research determined that exposure to inorganic arsenicals caused a significant increase in the total dry biomass production in the hydroponically grown marsh grass *Spartina alterniflora* [Carbonell *et al*., 1998]. Further investigation is required to help determine if the increase in the growth of the roots of common reed is a response to the exposure to CT tailings. Armstrong *et al*. [2010] evaluated diluted MFT with a gypsum amendment as well as simulated runoff water from dried MFT materials with a combination gypsum-lime amendment. Armstrong and colleagues [2010] found that both the diluted MFT and the simulated runoff water caused a significant decrease ($P < 0.05$) in the fresh weight of common reed. Interestingly, they also found that of the tailings treatments evaluated, the simulated runoff water from the gypsum-lime amended tailings had the highest concentration of naphthenic acids which could explain the reduction in fresh weight [Armstrong *et al*., 2010]. In the present experiment, no significant difference ($P < 0.05$) was found between the fresh weight of control common reed and common reed grown in nutrient media spiked with naphthenic acids, however there was a significant difference ($P < 0.05$) between the fresh weight of common reed grown in nutrient media spiked with naphthenic acids and the common reed grown in the two CT release water treatments (Figure 4.2). The common reed grown in the media spiked with naphthenic acids did not appear to have increased growth in the roots of the plants which may be a further indication that some constituent of the release water (other than the naphthenic acids) caused the increased growth observed in the roots of common reed. Furthermore,

common reed appears to be less tolerant to growing in nutrient media spiked with naphthenic acids than in CT release water.

Water chemistry was analyzed by the Saskatchewan Research Council (SRC) for oil sands tailings water samples (Table 4.1; Appendix A for complete results). Major ion analysis was conducted using inorganic chemistry and inductively coupled plasma mass spectrometry (ICP-MS). Linear regression was used to determine if relationships exist between the fresh weight of the plants over the 30 day experiment and the concentration of various ions in the CT reclaim water, with or without a gypsum amendment. Given the concentrations of ions detected in the samples, no significant relationships were detected between the fresh weight of either species over the 30 day experimental period and any of the parameters listed in Table 4.1. Furthermore, when compared to the quarter-strength Hoagland's nutrient media, it does not appear that the CT release water contains greater concentrations of nutrients that may be responsible for the increased growth. For example, the control media contains 53 mg/L nitrogen, 59 mg/L potassium, 55 mg/L calcium, and 12 mg/L magnesium [Hoagland & Arnon, 1950].

Table 4.1. Water chemistry data for Canmet oil sands tailings release water. Release water treated with gypsum is indicated as 'gypsum'. Release water without a gypsum amendment is indicated as 'no gypsum'. A value of '0' indicates that the ion was not detected at a level greater than 1 mg/L. Data are milligrams of ion per liter of water (n=1) (Appendix A).

Sample ID	HCO_3^-	CO_3^-	Cl^-	OH^-	NO_3^-	Ca^{2+}	Mg^{2+}	K^+	Na^+	SO_4^{2-}	PH
Gypsum	886	0	427	0	53	25	13	18	697	360	8.13
No gypsum	525	7	558	0	71	12	11	24	669	150	8.16

4.3.3 Water uptake

The water uptake data for this experiment are presented in Figure 4.3 (cattail) and Figure 4.4 (common reed). A significant reduction ($P < 0.05$) in the water uptake of cattail grown in CT release water (with and without gypsum) was observed for every five day period. With the exception of the measurements taken on Day 5, there were no significant differences ($P > 0.05$) found between the water uptake of cattail grown in CT release water with gypsum and the water uptake of cattail grown in CT release water without gypsum. The trend seen in Figure 4.3 indicates that the cattail are more tolerant to growing in CT without gypsum but the significance of this trend is masked by the high variability of the data.

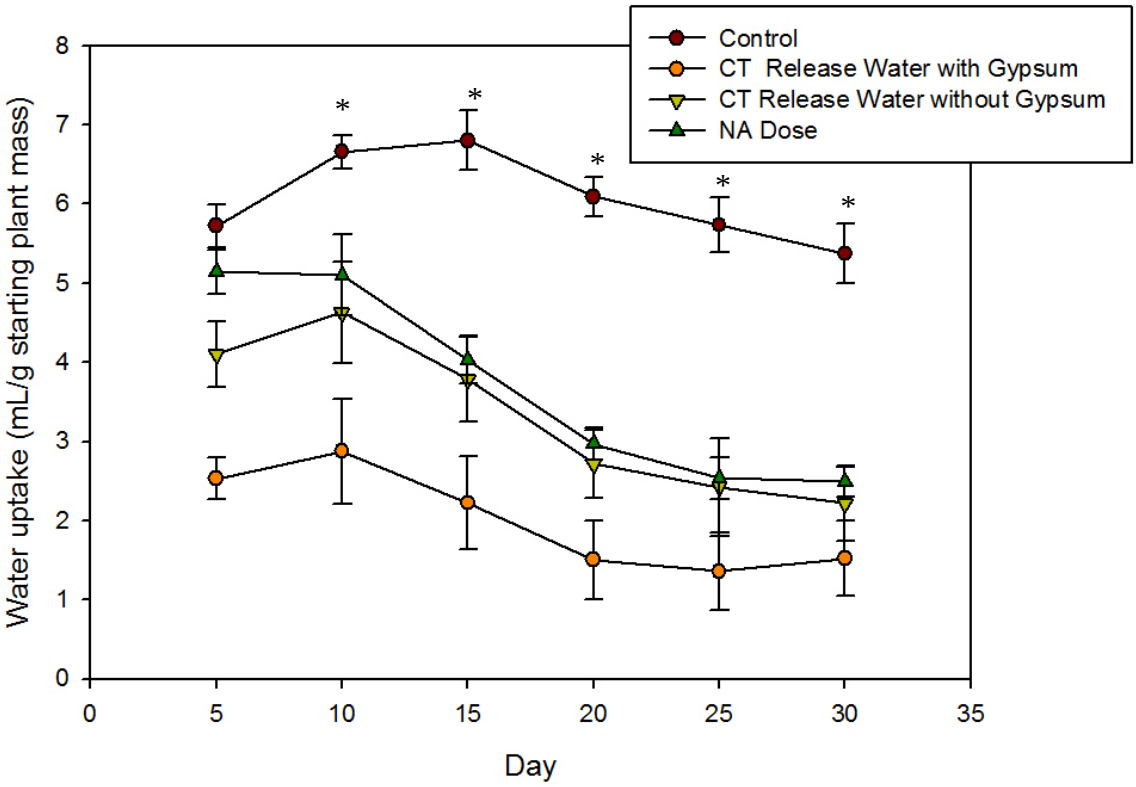

Figure 4.3. Cattail water uptake over the 30 day experiment evaluating the phytotoxicity of consolidated tailings and naphthenic acids. Data are the mean (n=4) volume of water taken up per gram of starting plant mass ± standard error. An asterisk (*) indicates a significant difference ($P < 0.05$) between the control group and all treatment groups.

With the exception of the Day 0 to Day 5 period, nutrient media spiked with naphthenic acids significantly reduced the water uptake of cattail ($P < 0.05$). Similar results have been observed previously whereby concentrations of naphthenic acids of 30 mg/L and 60 mg/L significantly altered water uptake in cattail [Armstrong *et al*., 2008].

The water uptake data for common reed is presented in Figure 4.4. A significant difference ($P < 0.05$) was found between the water uptake of control common reed and common reed grown in CT without gypsum for the majority of the experiment. It was observed that overall, common reed grown in CT release water without gypsum had greater rates of water uptake than control plants and plants grown in CT release water with gypsum. This trend is reflected in the fresh weight data whereby a substantial increase in fresh weight was observed in common reed grown in CT release water without gypsum. It appears that common reed is more tolerant to growing in CT release water without gypsum. For example, on Day 30 common reed grown in CT without gypsum took up 19.1 mL water per gram of starting fresh weight, whereby common reed grown in CT release water with gypsum took up 13.7 mL water per gram of starting fresh weight. It should be noted however that towards the midpoint of the experiment, the water uptake rates of common reed grown in CT release water with gypsum started increasing. It has been previously reported that other species of aquatic macrophytes are able to recover following exposure to phytotoxic chemicals [Teodorović *et al*., 2012] therefore it is possible that the increasing water uptake rates of common reed could be a possible sign of recovery.

No significant differences ($P < 0.05$) were found between the water uptake data for control common reed and common reed growing in nutrient media spiked with

naphthenic acids. These results are supported by those found by Armstrong *et al.* [2008] whereby nutrient media with a concentration of 30 mg/L naphthenic acids did not have an overall significant effect on the water uptake of common reed. It was found that a dose of 60 mg/L naphthenic acids did however have a significant effect on the water uptake of common reed [Armstrong *et al*., 2008].

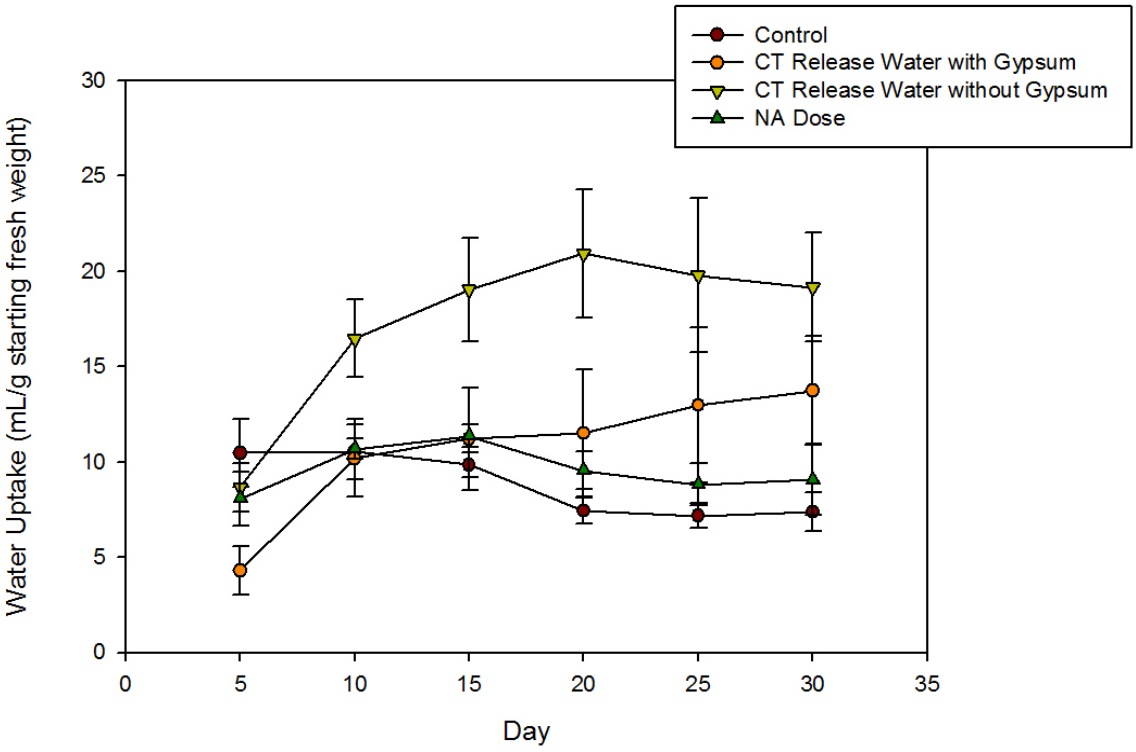

Figure 4.4. Common reed water uptake over the 30 day experiment evaluating the phytotoxicity of consolidated tailings and naphthenic acids. Data are the mean (n=4) volume of water taken up per gram of starting plant mass ± standard error. An asterisk (*) indicates a significant difference ($P < 0.05$) between the control group and all treatment groups.

4.4 Conclusions

Based on the results of the phytotoxicity experiments, it appears that the aquatic macrophytes common reed and cattail are most tolerant to growth in CT release water

without a gypsum amendment. Common reed appears to be more adaptable than cattail to growing in CT release water and nutrient media spiked with naphthenic acids and is therefore the stronger choice of the two species for use in oil sands reclamation strategies. As previously noted, further investigation is required to evaluate the cause of the increase in the growth of the roots of common reed grown in CT release water.

CHAPTER 5

THE NAPHTHENIC ACID MOLECULAR PROFILE

5.1 Introduction

Naphthenic acids are a complex mixture of organic acids that are a natural constituent of oil sands deposits [Quagraine *et al*., 2005]. Classical naphthenic acids are defined by the following formula, $C_nH_{2n+z}O_2$, where n is the carbon number, and Z describes the hydrogen deficiency that is a result of ring formation (where Z = zero or a negative integer) [Barrow *et al*., 2010; West *et al*., 2011]. Classical naphthenic acids can be arranged into categories based on their Z-groups whereby the absolute value of Z divided by two represents the number of rings. For example: structures are considered acyclic when Z equals 0, monocyclic when Z equals -2, bicyclic when Z equals –4, tricyclic when Z equals –6, and so on [Kannel & Gan, 2012]. The definition of naphthenic acids has recently been broadened to include the classically defined naphthenic acids (containing two oxygen atoms) as well as dicarboxylic and polycarboxilic acids, and O_x (where x = 1-6) containing species, along with other acid-extractable organics with aromatic functional groups, with or without nitrogen and sulfur atoms [Headley *et al*., 2009; Headley *et al*., 2011] (Figure 5.1).

Naphthenic acids can enter the aquatic environment through the natural erosion of oil deposits as well as through effluent discharges and leakages from oil sands tailings ponds [Kannel & Gan, 2012]. Naphthenic acids act as surfactants, possessing a hydrophyllic end and a hydrophobic end [Armstrong, 2008]. The solubility of naphthenic acids in water is pH dependent, with solubility values ranging from 0.070 mg/mL at pH 0.91 and 5.04 mg/mL at pH 9.16 [Armstrong, 2008]. Due to the alkaline pH of oil sands

tailings, naphthenic acids found within oil sands tailings are predominantly in their ionized form as naphthenate salts, which are water-soluble [Headley & McMartin, 2004]. Once in the aquatic environment, some components of naphthenic acids have limited bioavailability due to low solubility and relatively strong sorption to soil [Quagraine *et al*., 2005]. Naphthenic acids are soluble in organic solvents [Headley & McMartin, 2004]. Due to the complexity of naphthenic acids, and the different processing techniques and tailings ponds management schemes used by different oil sands operators [Kasperski & Mikula, 2011], the physical and chemical composition of each naphthenic acid mixture may vary [Kannel & Gan, 2012].

In general, the background levels of naphthenic acids in river waters of northern Alberta are below 1 mg/L [Headley *et al*., 2011; Ross *et al*., 2012]. Due to the repeated recycling of oil sands water from tailings, over time naphthenic acids can become concentrated in tailings ponds waters [Quagraine *et al*., 2005], and some process waters may contain concentrations as high as 110 mg/L [Headley *et al*., 2011]. Although the principal toxic components have yet to be identified [Headley *et al*., 2011] as a group, naphthenic acids have been identified to contribute to the toxicity of oil sands tailings [Allen, 2008].

The objectives of this study were to determine the naphthenic acid molecular profile for the naphthenic acid mixture contained within Shell's Muskeg River Mine mature fine tailings (MFT), and the release and runoff water associated with Shell's atmospheric fines drying (AFD) process. This study also investigated whether or not the Shell AFD process alters the composition of the naphthenic acid mixture found in oil

sands tailings water and whether any compositional changes to the naphthenic acids occurred over the 30 day hydroponic studies conducted.

5.2 Materials and methods

5.2.1 Sample collection

During the 30 day hydroponic studies (Described in Sections 2.2.2, 3.2.1, and 4.2.3) one-two mL sample of tailings water was collected from each jar using a Pasteur pipette, on Day 0 and Day 30 of the experiment to evaluate any changes to the composition of the naphthenic acids over the course of the experiments. One 2 mL sample of naphthenic acid extract (Section 4.2.1) was also collected for analysis.

5.2.2 Sample preparation

In order to account for background ion interference [Armstrong *et al*., 2009], and to concentrate the polar organic constituents, hydroponic samples were cleaned prior to injection using 6 mL Isolute SPE+ solid phase extraction (SPE) cartridges [Armstrong *et al*., 2008]. Each 1.5 mL hydroponic sample was diluted with 6 mL of milli-Q water, then acidified using 0.5 mL of formic acid to a pH of 2. For each sample, a 200 mg Isolute ENV + SPE cartridge (Biotage, Charlotte, NC) was preconditioned by first running 6 mL of milli-Q water through the cartridge, followed by 6 mL of acetonitrile, then an additional 6 mL of milli-Q water. Both acetonitrile and milli-Q water were passed through the column at a flow-rate of approximately 5 mL/min. Each acidified sample was then drawn through an SPE cartridge under vacuum suction at a flow rate of approximately 1 mL/min. 5 mL of milli-Q water was then percolated through each column at a rate of approximately 1 mL/min.

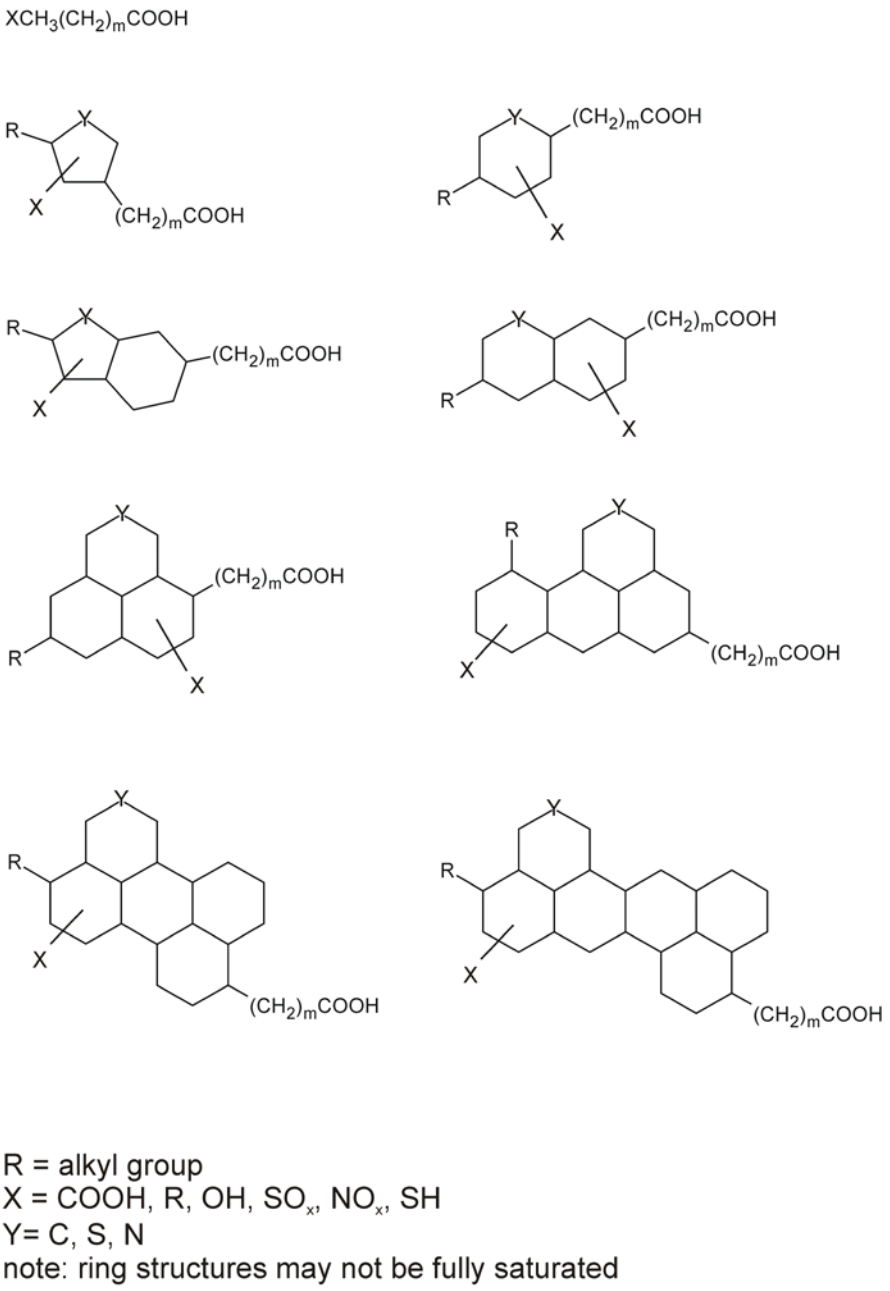

Figure 5.1. Representative molecular structures of naphthenic acid fraction components as outlined by the most recent naphthenic acid definition, which includes the classical naphthenic acids [Headley *et al*., 2011].

Finally, the organic acids adsorbed to the SPE cartridge were eluted into glass collection tubes using 6 mL of acetonitrile under vacuum suction at a flow rate of approximately 1 mL/min. Samples were then evaporated to dryness under a nitrogen gas stream. Once completely dry, samples were re-dissolved in 1 mL of 50:50 acetonitrile/water containing 0.1 % ammonium hydroxide (NH_4O_4).

5.2.3 Low resolution mass spectrometry

Analyses of tailings water samples collected during the AFD release water experiments were performed using electrospray ionization mass spectrometry (ESI-MS) [Headley *et al.*, 2002] at the National Hydrology Research Center in Saskatoon, Saskatchewan, Canada. Mass spectromic analysis was conducted with a Quattro Premier triple quadrupole mass spectrometer (Waters/Micromass, UK) equipped with an ESI interface operating in the negative ion mode [McMartin *et al.*, 2004]. Detailed instrument settings may be found in Appendix B.

5.2.4 High resolution mass spectrometry

Analyses of tailings water samples collected during the AFD release and runoff water experiments were performed using a LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific) using electrospray ionization in the negative ion mode at the National Hydrology Research Center in Saskatoon, Saskatchewan, Canada. Samples were analyzed in full scan with m/z range of 100-600 and the resolution was set to 100,000. Naphthenic acid concentrations were determined by comparison to a pre-defined 5-point regression of naphthenic acids at known concentrations. Xcalibur version 2.1 software

(Thermo Fisher Scientific) was used for data acquisition, instrument operation, and quantitative data analysis. Class distribution was determined using acquired accurate mass data and Composer version 1.0.2 (Sierra Analytics, Inc.). Detailed instrument settings may be found in Appendix C. The variability of analysis was determined by analyzing three replicate samples and calculating the relative standard deviation (RSD).

5.3 Results and discussion

5.3.1 Low resolution mass spectrometry

For the first time (to the author's knowledge) a naphthenic acid molecular profile was determined for Shell Canada tailings pond water (Figure 5.2A 5.2B). The naphthenic acid molecular profiles were produced by plotting low-resolution mass spectral data using a 3D coordinate system of the percent abundance of *classical* naphthenic acids according to the carbon number (n) and Z-family ($C_nH_{2n+z}O_2$). Small fluctuations in percent abundance are evident (Figure 5.2A and 5.2B), but the constituents of the mixtures appear to remain the same (i.e. the –Z family and carbon number (n) ranges are the same for both mixtures). Statistical analyses of the low resolution data failed to provide consistent evidence that there are statistically significant differences between the percent abundances of each –Z family and carbon number combinations for different tailings water types (data not shown). For example, statistically significant differences were found between the molecular profiles of the naphthenic acids found in Shell AFD Cell 8 release water and Shell AFD composite release water. The processing of these two waters only differs by the slopes of the cells that the release waters were collected from. It can therefore be concluded that the low resolution mass spectrometry is unable to detect any differences between the naphthenic acids found in MFT and AFD release

water. Furthermore, it can also be concluded that when using low resolution mass spectrometry, no detectable changes were found between the naphthenic acid mixture observed in Shell oil sands tailings and those found in oil sands tailings produced by a non-Shell oil sand operator (Figure 5.2C). This observation indicates that differences in oil sands processing techniques and tailings pond management between Shell and the non-Shell oil sands operator do not appear to have caused any detectable changes to the naphthenic acids present in tailings.

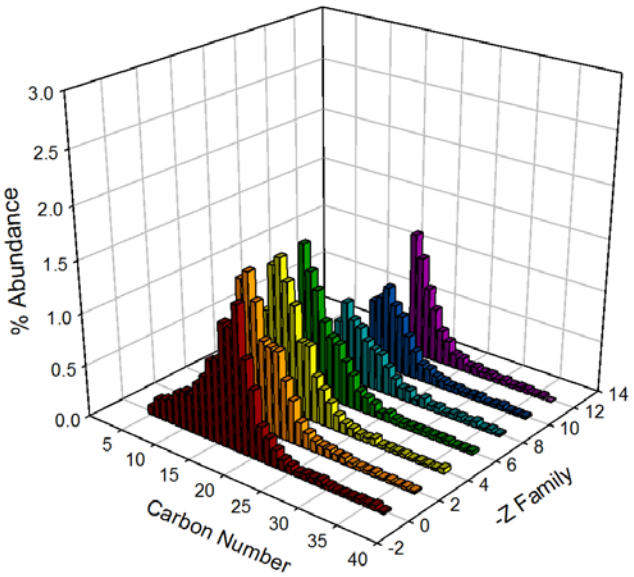

Figure 5.2A. Naphthenic acid molecular profile derived from the naphthenic acids found in Shell mature fine tailings (MFT). Molecular profiles are percent abundance of naphthenic acids vs. carbon number and Z family on Day 0 of the hydroponic experiment (n=3).

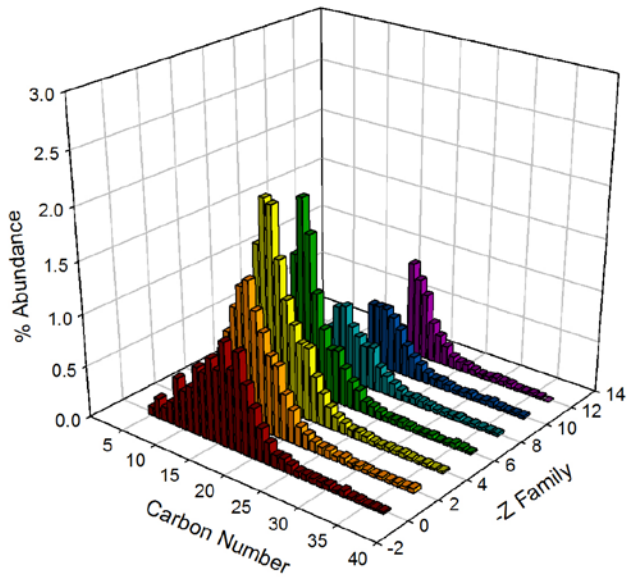

Figure 5.2B. Naphthenic acid molecular profile derived from the naphthenic acids found in Shell atmospheric fines drying (AFD) release water. Molecular profiles are percent abundance of naphthenic acids vs. carbon number and Z family on Day 0 of the hydroponic experiment (n=3).

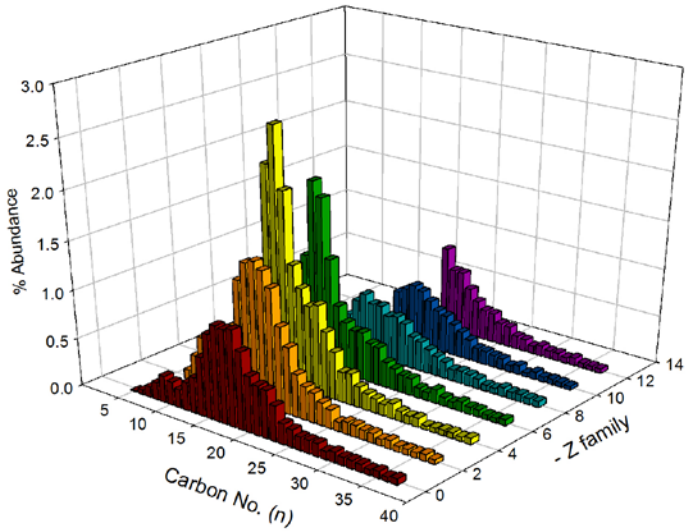

Figure 5.2C. Naphthenic acid molecular profile derived from the naphthenic acids found in the tailings of a non-Shell oil sands operator. Molecular profiles are percent abundance of naphthenic acids vs. carbon number and Z family on Day 0 of the hydroponic experiment (n=3).

The concentrations of naphthenic acids detected in the MFT reclaim water and the AFD release water detected using low resolution mass spectrometry are reported in Table 5.1. When examining the naphthenic acid concentrations found in the different oil sands water, it is apparent that the AFD release waters contain higher levels of naphthenic acids than MFT reclaim water. The smaller concentration of naphthenic acids detected in the MFT is likely due to the biodegradation of naphthenic acids in the MFT reclaim water over time. The AFD water was collected daily from the weirs at the base of the cells therefore there was limited opportunity for biodegradation to occur. Han *et al*. [2008] report that selective removal of naphthenic acids with lower molecular masses, where n is less than or equal to 21, does occur, however, the mechanism by which the microbial degradation of naphthenic acids occurs is poorly understood. Following decades of microbial degradation, a persistent fraction of naphthenic acids remains, with the lowest concentration of naphthenic acids in aged tailings reported as 19 mg/L [Quagraine *et al*., 2005; Holowenko *et al*., 2002]. Han *et al*. [2008] report that increased cyclinization (Z) indicates a decreased biodegradation rate for naphthenic acids, while the carbon number, n, has little effect.

Table 5.1. Naphthenic acid concentrations in mature fine tailings (MFT) and atmospheric fines drying (AFD) release water determined using low resolution mass spectrometry. Data are the mean (n =6) naphthenic acid concentrations (mg L^{-1}) ± standard error in tailings water on Day 0 of the experiment, for samples taken from hydroponic vessels containing both cattail and common reed.

Tailings Water Type	Naphthenic Acids, Day 0 (mg/L; ± SE)
MFT	24.7 (2.7)
Cell 8 Release Water (AFD)	38.7 (3.3)
Composite Release Water (AFD)	45.2 (1.0)

5.3.2 High resolution mass spectrometry

Due to the complexity of the naphthenic acid mixture, the quantitative mass spectrometry data generated in this study was used only to identify visible trends (i.e. no statistical analyses were undertaken). The concentrations of naphthenic acids detected in hydroponic samples taken throughout the naphthenic acid dosing study are represented in Figure 5.3. During this study, each vessel contained 2.5 L of nutrient media, which was spiked with 25.5 mL of naphthenic acid extract to achieve a naphthenic acid concentration of approximately 40 mg/L (Section 4.2.3). The concentrations of naphthenic acids in samples taken from vessels planted with both species was observed to decrease from Day 0 to Day 5 of the experiment, then stabilize over the remaining 25 days. As reported by Armstrong [2008], naphthenic acids did not appear to dissipate over the course of the study. It should be noted however, that low-resolution mass spectrometry (as used in Armstrong [2008]) is unable to resolve the contribution of some compounds, for example fatty acids (Z=0) from the plants, which may mask the dissipation of naphthenic acids by increasing the overall concentration of 'naphthenic acids' [Headley *et al*., 2009].

It appears that the measured concentration of naphthenic acids in the samples collected on Day 0 from vessels planted with common reed (Figure 5.3) is much higher than the applied dose. Hydroponic media was spiked with enough naphthenic acid extract to achieve a nominal concentration of 40 mg/L, therefore an approximate starting concentration of over 60 mg/L, that dissipates down to approximately 43 mg/L in a period of five days demonstrates the semi-quantitative nature of high resolution mass spectrometry data. The semi-quantitative characteristic of high resolution naphthenic acid

data is attributable to some compounds not ionizing as well as others and also by the suppression or enhancement of some constituents by the sample matrix [as reviewed by Headley *et al*., 2009].

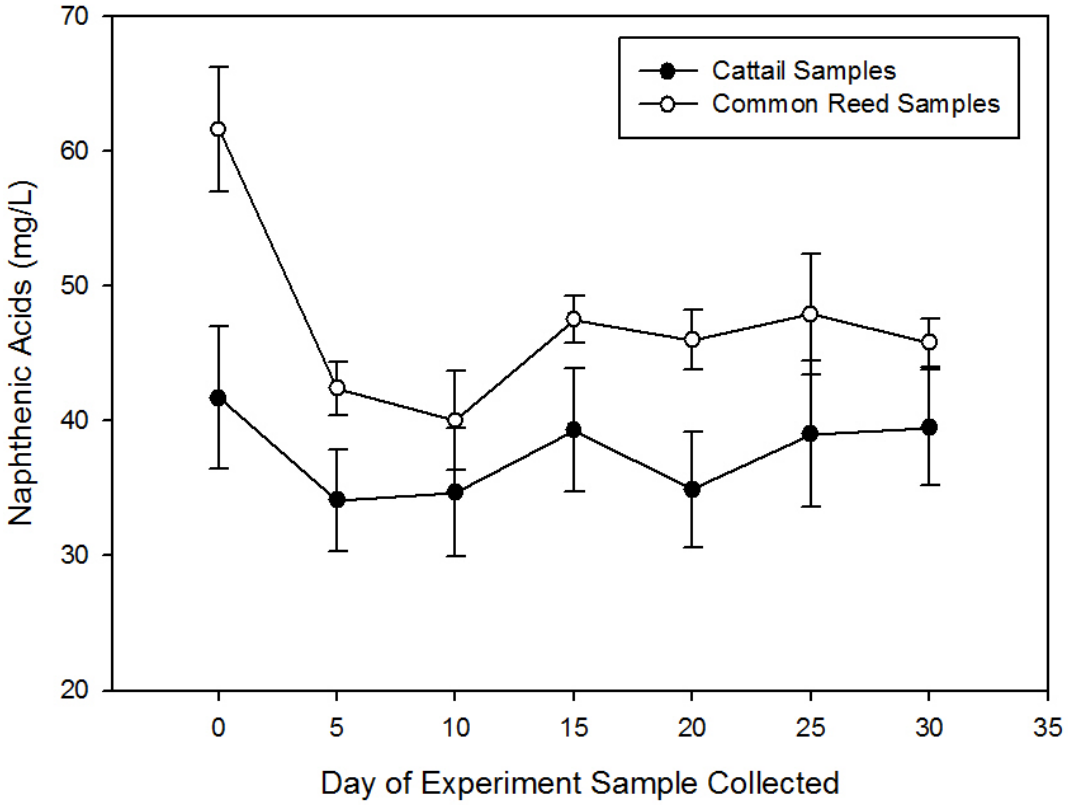

Figure 5.3. Naphthenic acid concentrations (mg/L) detected using high resolution mass spectrometry over the 30 day hydroponic naphthenic acid dosing study. Data are the mean (n=4) naphthenic acid concentrations ± standard error detected in hydroponic media for each day of the experiment the sample was collected. The starting concentration for this study was 40 mg/L.

In continuation from Section 5.3.1., data produced using high-resolution mass spectrometry was used in order to determine if Shell AFD processing affects the composition of the naphthenic acids found in oil sands tailings. Four types of Shell materials were analyzed for naphthenic acids: mature fine tailings (MFT), water released

from AFD Cell 8 during the initial water collection when AFD materials were first deposited onto the cells at the AFD pilot site, runoff water collected from Cell 8 during the spring snow melt, and a naphthenic acid extract prepared from Shell MFT using the techniques described in Section 4.2.1. The compound class molecular distributions for these four materials are represented in Figure 5.4A (nitrogen species) and Figure 5.4B (oxygen species).

Naphthenic acids are primarily represented by the O_2 series ($C_nH_{2n+z}O_2$). Therefore, using high-resolution mass spectrometry, the naphthenic acid mixture was further characterized by breaking down the O_2 series by the -Z distributions (Figure 5.5). The major differences in the composition of the naphthenic acids appears to be in the abundance of the Z = -4 and -6 species. In MFT reclaim water, the most abundant naphthenic acid is the tricyclic (Z = -6) family, followed by the bicyclic (Z = -4) family. In the release water, runoff water, and the naphthenic acid extract, the most abundant naphthenic acid is the bicyclic (Z = -4) family, followed by the tricyclic (Z = -6) family. This shift in percent abundance could be due to biodegradation. It has been demonstrated that lower molecular mass naphthenic acids (less negative Z number) are more susceptible to aerobic microbial processes, demonstrating selective removal that results in an increase in the relative concentration of higher molecular mass (more negative Z number) naphthenic acids that are less susceptible to biodegradation [Holowenko *et al*., 2002; Scott *et al*., 2005; Clemente *et al*., 2004; Biryukova *et al*., 2007].

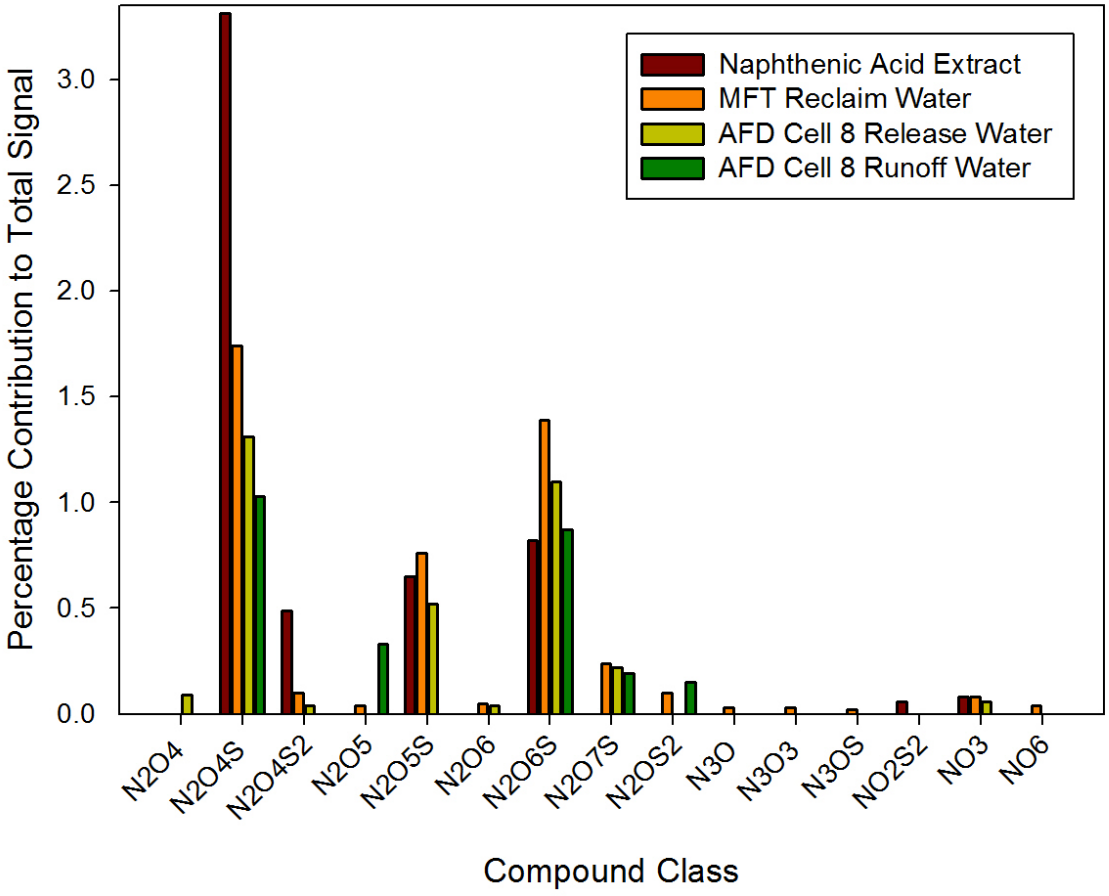

Figure 5.4A. Plot of the distribution of nitrogen species classes observed in the mass spectra for Shell naphthenic acid containing materials. Data are the percentage contribution to total mass spectral signal measured for each compound class detected (n=1).

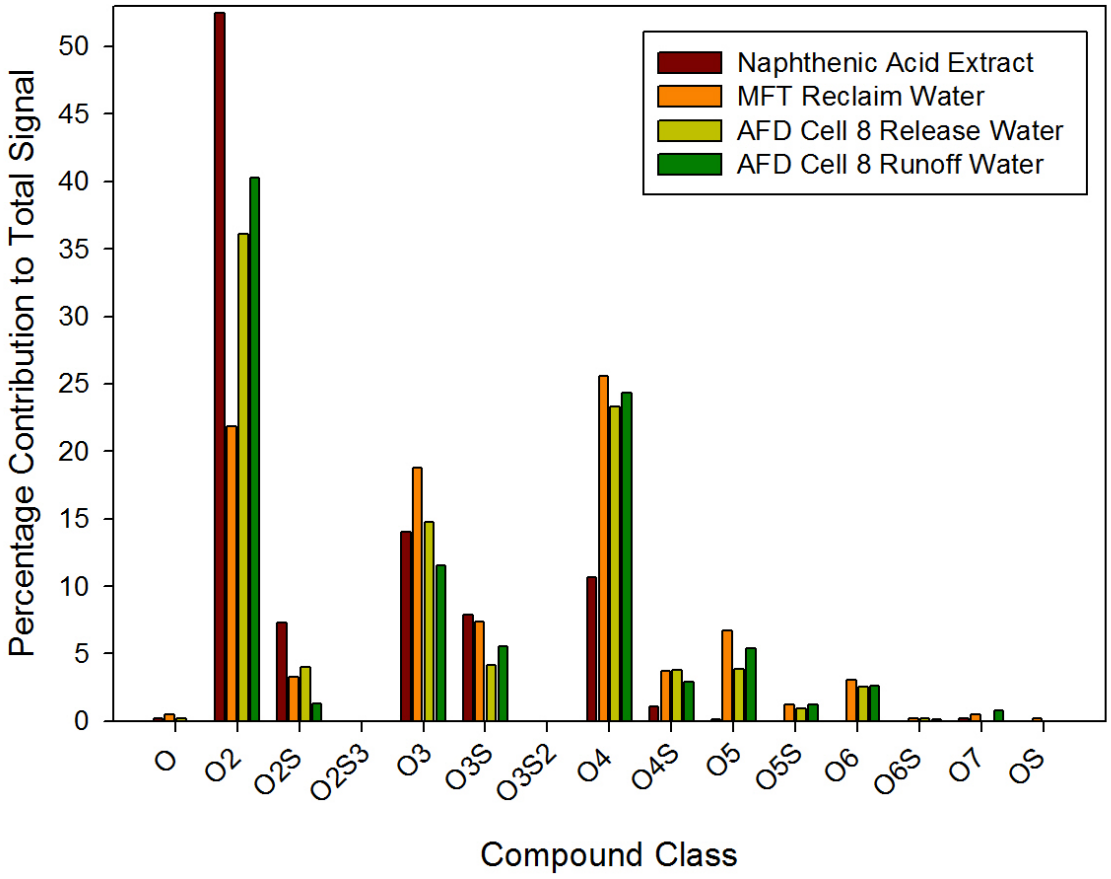

Figure 5.4B. Plot of the distribution of oxygen species classes observed in the mass spectra for Shell naphthenic acid containing materials. Data are the percentage contribution to total mass spectral signal measured for each compound class detected (n=1).

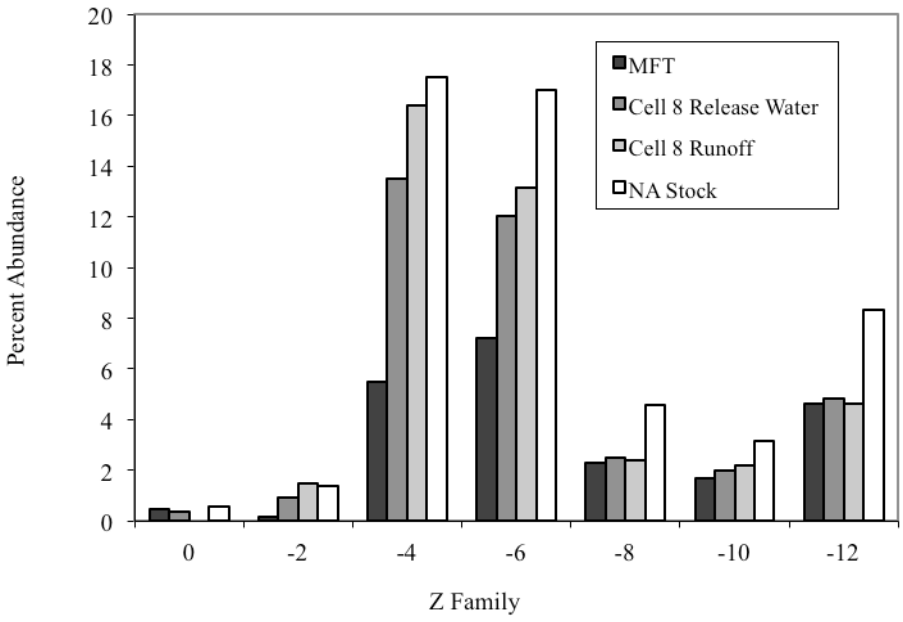

Figure 5.5. The O_2 series distribution for Shell naphthenic acid containing materials. Data are the Z family distributions within the O_2 class as percent abundance of the total signal (n = 1).

The distribution data were further characterized by breaking down the O_x series and examining the differences from Day 0 to Day 30 of the phytotoxicity experiments. When examining the distribution data for the O_2 series (Z distribution), plant species differences were detected. For example, from Day 0 to Day 30, there was an increase in the O_2 species in samples taken from vessels planted with common reed (Figure 5.6). However, the same trend was not seen in samples taken from vessels planted with cattail. In the cattail samples, a decrease in the O_2 species was observed (Figure 5.7).

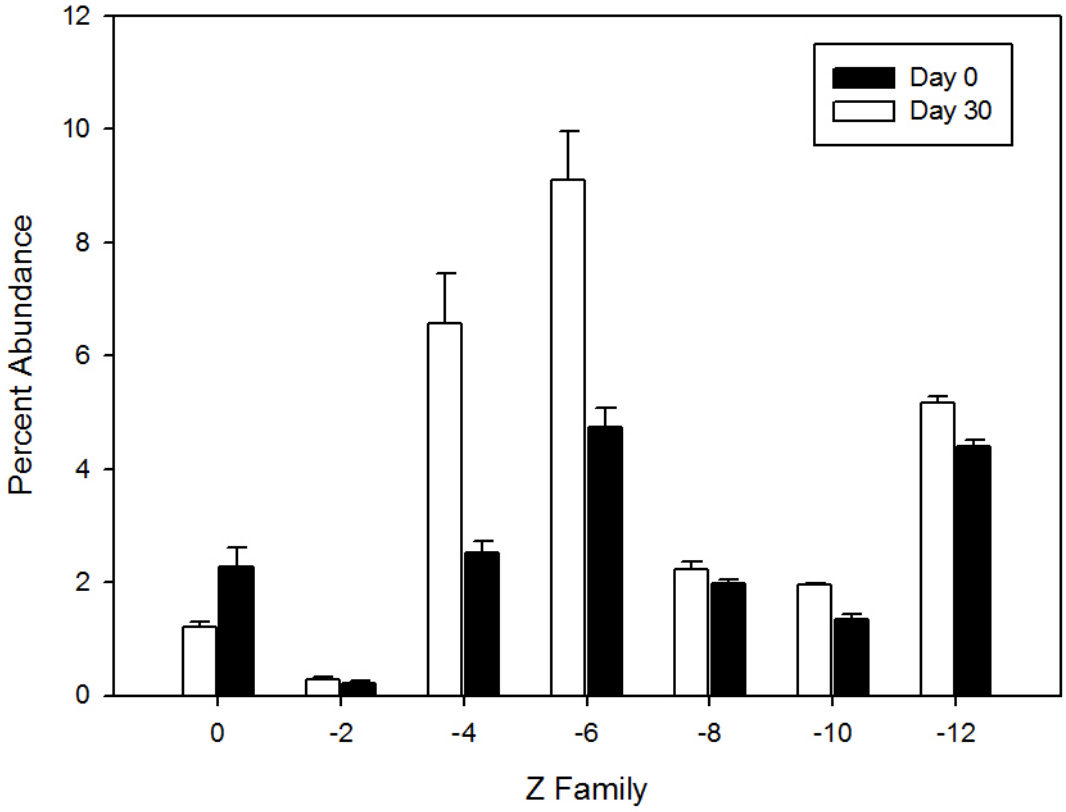

Figure 5.6. The O_2 series distribution for hydroponic atmospheric fines drying (AFD) release water samples taken on Day 0 and Day 30 of the hydroponic experiment from vessels planted with common reed. Data are the Z family distributions within the O_2 class as percent abundance of the total signal (n = 3; RSD 16.1%).

The breakdown of the O_2 series for release water samples taken from vessels planted with common reed revealed increases in the percent abundance for all of the classes detected (Z = 0 to -12) except for Z = 0 (Figure 5.6). Most notably, there were large increases in the Z = -4, and -6 classes. Kim *et al*. [2005] conducted studies using ultra-high Fourier transform ion cyclotron resonance mass spectrometry to observe the changes in the composition of oils during anaerobic biodegradation. The researchers observed that during advanced stages of microbial degradation, O_2 species with Z = -4, -6, and –8 are preferentially produced. While it does not appear that common reed is able

to dissipate naphthenic acids, there appears to be a shift in the composition of the acids present, which may be due to microbial degradation. Another explanation for an increase in O_2 species may a phytotoxic response whereby the plants are releasing O_2 classes of compounds.

Headley *et al*. [2009] detected fatty acids released by cattail exposed to naphthenic acids, and it appears that common reed showed a similar response during this study. It appears as though the potential phytotoxic response of common reed is more prominent than that of cattail. Interestingly, during the hydroponic phytotoxicity experiments (detailed in Chapters 2, 3, and 4) it was found that common reed was less susceptible to the phytotoxic effects of oil sands tailings containing naphthenic acids. Further investigation is required to determine if the release of these compounds is a mechanism by which common reed is able to adapt to growing in oil sands tailings.

Headley *et al*. [2009] compared the dissipation of naphthenic acids in samples collected from hydroponic vessels planted with cattail and samples collected from unplanted hydroponic vessels. It was found that vessels planted with cattail showed evidence of dissipation of naphthenic acids (O_2 series decreased). In the current study, the examination of the breakdown of the O_2 series for release water samples taken from vessels planted with cattail indicated that there were small decreases in percent abundance for all of the classes detected (Z = 0 to -12) except for Z = -2 where there was a small increase (Figure 5.7). These findings support Headley *et al*. [2009] where evidence of naphthenic dissipation by cattail was observed. This decrease in the O_2 series in the cattail samples provides an explanation for the phytotoxicity observed during the hydroponic experiments. Based on the high resolution data, it appears at though cattail

are able to take up the O_2 compounds, and during the hydroponic experiments, cattail were more susceptible to the phytotoxic effects of oil sands tailings water. However, it does not appear that common reed is able to take up the O_2 compounds, and common reed appeared to be able to adapt to growing in oil sands tailings water.

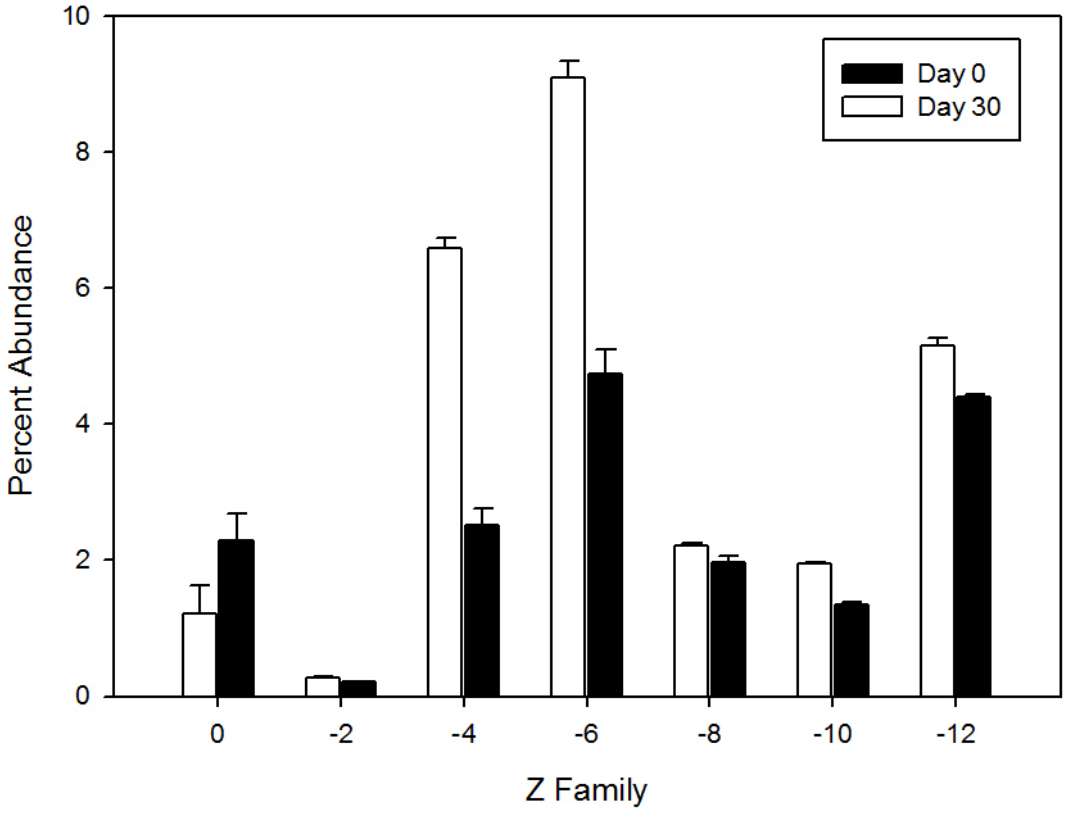

Figure 5.7. The O_2 series distribution for hydroponic atmospheric fines drying (AFD) release water samples taken on Day 0 and Day 30 of the hydroponic experiment from vessels planted with cattail. Data are the Z family distributions within the O_2 class as percent abundance of the total signal (n = 3; RSD 16.1%).

While it was not experimentally determined whether or not microbial degradation or abiotic losses of naphthenic acids to the hydroponic system occurred, previous studies using abiotic and biotic hydroponic controls have shown that the dissipation of ionized

naphthenic acids observed was due to the presence of aquatic plants [Armstrong, 2008]. However, the mechanism by which this dissipation occurred is unknown [Armstrong, 2008].

Figure 5.8 presents the distribution of the O_3 species in samples taken from vessels planted with common reed. From Day 0 to Day 30, there were notable increases in the Z= -2, -4, and –6 species and decreases in the Z= -8 and –12 classes. Similar trends were observed in the samples collected from vessels planted with cattail, however to a much lesser extent (Figure 5.9).

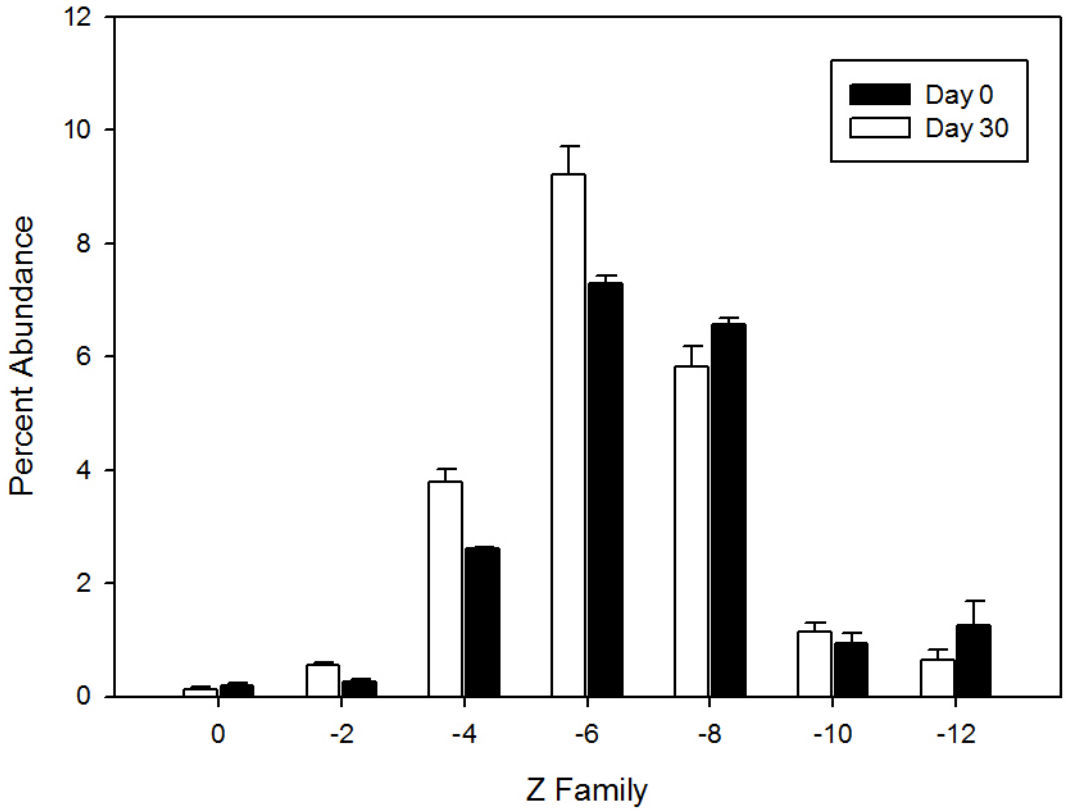

Figure 5.8. The O_3 series distribution for hydroponic atmospheric fines drying (AFD) release water samples taken on Day 0 and Day 30 of the hydroponic experiments from vessels planted with common reed. Data are the Z family distributions within the O_3 class as percent abundance of the total signal (n = 3; RSD 16.1%).

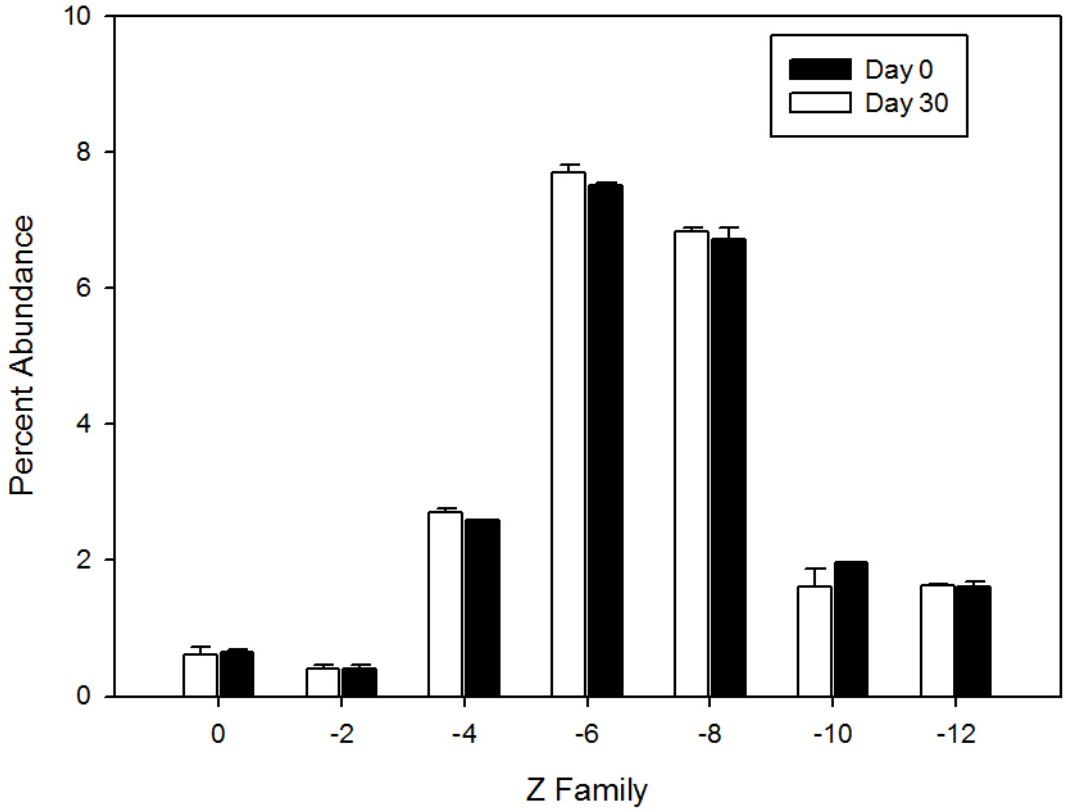

Figure 5.9. The O_3 series distribution for hydroponic atmospheric fines drying (AFD) release water samples taken on Day 0 and Day 30 of the hydroponic experiment from vessels planted with cattail. Data are the Z family distributions within the O_3 class as percent abundance of the total signal (n = 3; RSD 16.1%).

Headley *et al*. [2009] concluded that the O_3 species present in hydroponic media spiked with naphthenic acids were not susceptible to dissipation by cattail, and this conclusion is supported with the data collected from cattail samples during this study.

The analysis of the O_4 classes produced similar results to those of the O_3 classes, for samples collected from vessels planted with both species (Figure 5.10 and Figure 5.11). In common reed, decreases in the more negative Z value compounds may indicate that these compounds are more susceptible to dissipation by common reed and to microbial degradation. It should be mentioned however, that in cattail, it appears as

though the changes in naphthenic acid composition seem to be more subtle in the O_4 series, indicating a resistance of the O_4 class to dissipation by cattail.

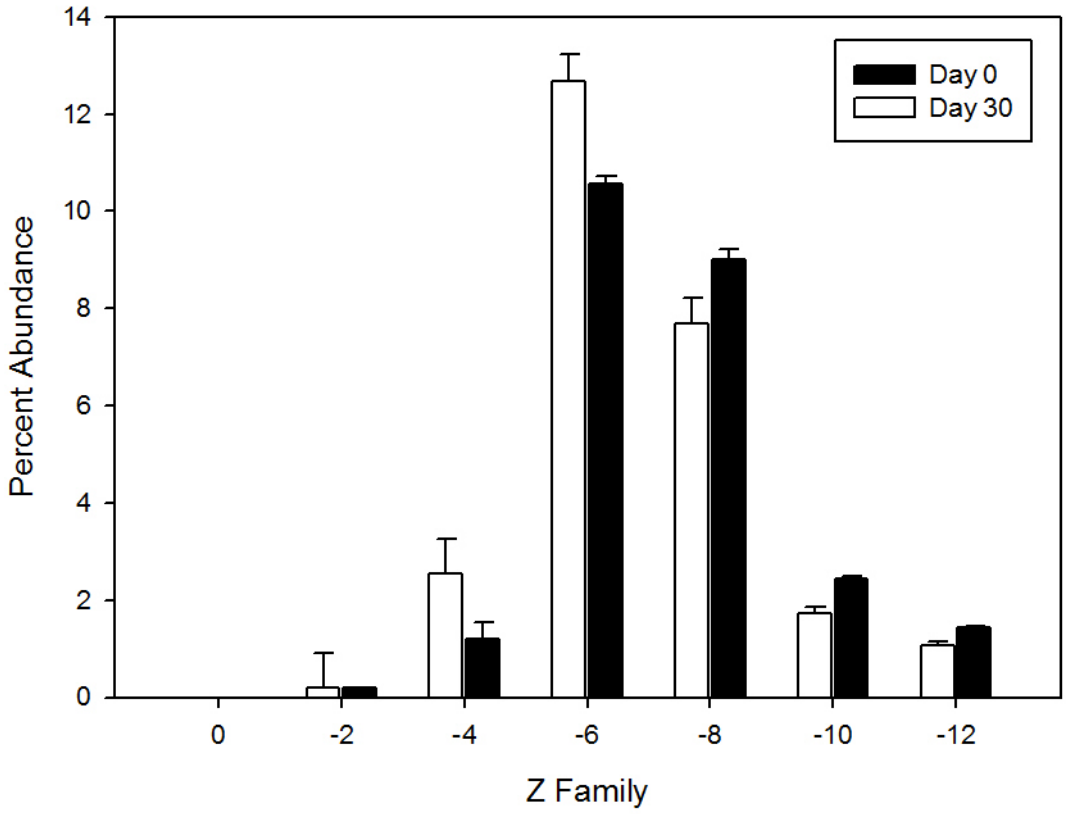

Figure 5.10. The O_4 series distribution for hydroponic atmospheric fines drying (AFD) release water samples taken on Day 0 and Day 30 from vessels planted with common reed. Data are the Z family distributions within the O_4 class as percent abundance of the total signal (n = 3; RSD 16.1%).

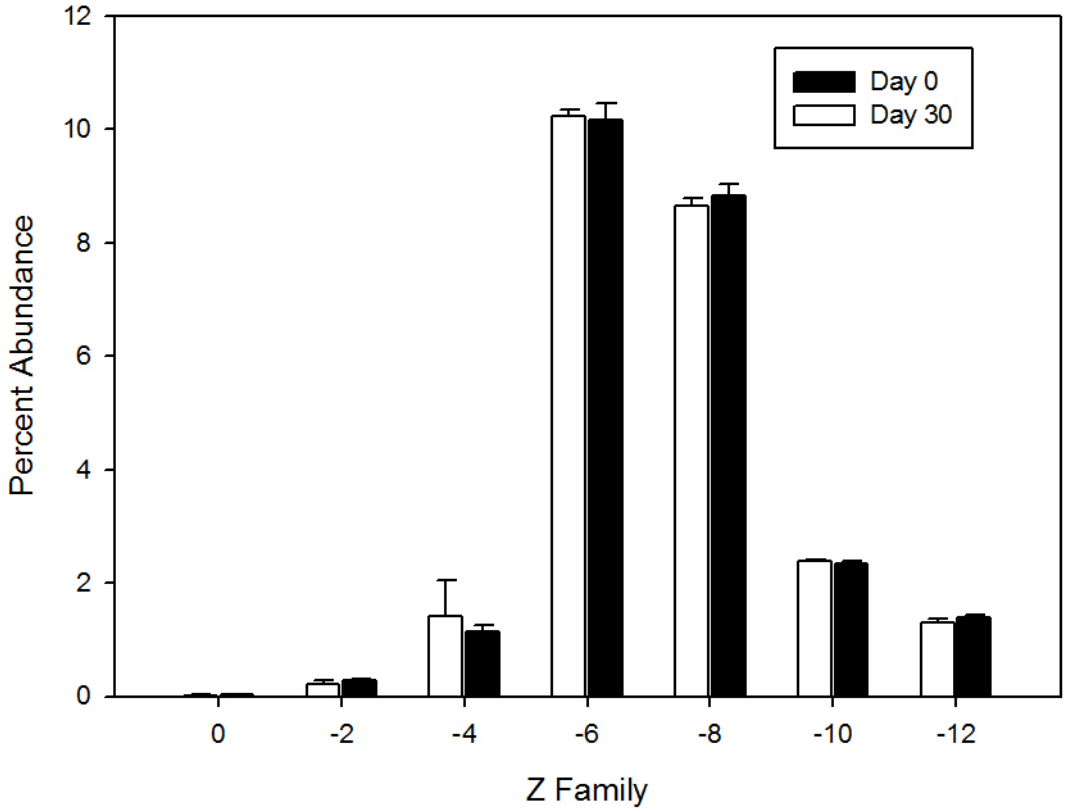

Figure 5.11. The O_4 series distribution for hydroponic atmospheric fines drying (AFD) release water samples taken on Day 0 and Day 30 from vessels planted with cattail. Data are the Z family distributions within the O_4 class as percent abundance of the total signal (n = 3; RSD 16.1%).

5.4 Conclusions

Using a combination of low and high-resolution mass spectrometry, it was determined that AFD processing does not appear to strongly affect the range of compounds present in the naphthenic acid mixture. Small changes detected using high-resolution mass spectrometry may be explained by microbial biodegradation.

This study has provided possible evidence of plant-mediated changes to naphthenic acids. It appears as though cattail is able to dissipate the naphthenic acids found in Athabasca oil sands tailings, more specifically, the O_2 species. However it does

not appear that common reed is able to aid in the dissipation of naphthenic acids. In regards to the increased abundance of fatty acids detected, high-resolution mass spectrometry has provided clues of a possible phytotoxic response. Further investigation is required to confirm if the plants are releasing these compounds as an adaptation to growing in oil sands tailings. This study suggests that a combination of microbial degradation and the use of cattail may be a useful tool in the reclamation strategies involving oil sands tailings.

CHAPTER 6

GENERAL CONCLUSIONS

6.1 Introduction

In order to fulfill the research objectives outlined in Section 1.4, hydroponic experiments using cattail (*Typha latifolia* L.) and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) were conducted to evaluate the phytotoxicity of the release and runoff waters formed in connection with the atmospheric fines drying (AFD) process. For comparison, the phytotoxicity of mature fine tailings, release water from tailings treated with gypsum, and the phytotoxicity of adding a naphthenic acid extract to hydroponic growth media were also evaluated. Using mass spectrometry, the naphthenic acid molecular profile for AFD materials was determined, and process and plant mediated changes to the compounds that contribute to the naphthenic acid molecular profile were investigated.

The present body of research provides important insight into the use of cattail and common reed as part of reclamation strategies of oil sands surface mines and tailings ponds. Both species are fast growing native perennial wetland plants and are tolerant of a broad range of growing conditions [Mitich, 2000; Mal & Narine, 2004; United States Department of Agriculture, 2010]. In providing water and wind erosion control and vital habitat for fish and wildlife [Oil Sands Wetlands Working Group, 2000], wetlands are an ideal component of the reclamation strategies. The present studies demonstrate that common reed, and to some extent, cattail, may be successfully incorporated into the reclaimed landscape.

6.2 The phytotoxicity of AFD release water on cattail and common reed

Objective: *To determine the phytotoxicity of release waters formed in connection with tailings produced by the atmospheric fines drying (AFD) process compared to reclaim water produced from traditional mature fine tailings (MFT).*

The 30 day hydroponic experiments conducted revealed that the release water collected from the weirs at the base of the AFD cells did not cause an increase in phytotoxicity to cattail and common reed when compared to the phytotoxic effects of MFT reclaim water. This indicates that the polyacrylamide added to MFT in the AFD process does not increase the phytotoxicity of the tailings to cattail and common reed. Analytical analysis of the AFD release water using LC-MS indicated levels of acrylamide that were below the instrument detection limits of 1 µg/L.

AFD release water and MFT reclaim water both significantly reduced the whole plant fresh weight and water uptake rates of cattail. The visible phytotoxic effects in cattail were also more pronounced than those in common reed. Common reed appears to be more readily able to adapt to growing in AFD release water and MFT reclaim water. This is indicated by there being no significant effect of release or reclaim water on the fresh weight of common reed, and by the lack of visible phytotoxicity. Throughout the experiment, common reed plants continued to produce new shoots and expand their root systems. Although water uptake of common reed was significantly decreased by the release and reclaim water, it appears as though the water uptake reached a plateau, and did not cease all together, which may be a possible indication of adaptation.

6.3 The phytotoxicity of AFD runoff water on cattail and common reed

Objective: *To determine the effects of over wintering on AFD deposits by testing the phytotoxic effects of snowmelt runoff water from AFD deposits compared to similar waters produced from MFT deposits.*

Spring snowmelt runoff water collected from the AFD cells following a winter under snow cover was used to determine the over wintering ability of the AFD deposits. The 30 day phytotoxicity experiments revealed an improvement in the phytotoxicity associated with the AFD release water evaluated the previous fall. In cattail, fewer visible signs of toxicity were observed, no significant difference in water uptake was found when compared to control cattail, and an improvement in water uptake was seen when compared to the cattail grown in AFD release water. In common reed, no visible signs of phytotoxicity were observed and surprisingly, common reed growing in AFD runoff water experienced greater fresh weight and water uptake than common reed grown in control media. It appeared as though the majority of the increase in fresh weight was due to an increase in the overall size of the roots of common reed. It was hypothesized that increased nutrient concentrations were responsible for the increase in growth; however the water quality data collected does not appear to support this hypothesis and further investigation is required to determine if the increase in root growth is a phytotoxic response.

Due to dilution, it was expected that AFD runoff water would be less phytotoxic to cattail and common reed than AFD release water. However, the water evaluated during

this study was collected near the end of the spring thaw and it has been indicated that the first 30% of snowmelt water contains 50-80% of the pollutant load [Johannessen & Henriksen, 1978]. In order to achieve a more realistic seasonal representation of the potential phytotoxicity of runoff water from the AFD deposit, water samples should be collected throughout the spring thaw. Therefore, future studies should include hydroponic experiments conducted where the spring runoff is simulated by maintaining the volume of runoff water in the growing vessels with water collected from the AFD deposits on Days 5, 10, 15, 20, and 25 of the spring runoff.

6.4 The Phytotoxicity of Consolidated Tailings Release Water and Nutrient Media Spiked with Naphthenic Acids on Cattail and Common Reed

Objective: *To determine the phytotoxicity of tailings treated with gypsum and hydroponic growth solution spiked with a naphthenic acid extract.*

6.4.1 Consolidated tailings release water

Hydroponic experiments were conducted to evaluate the potential phytotoxicity of consolidated tailings (CT) release water to cattail and common reed. The CT process is an older method that has been used to speed up the settling of fines suspended in MFT. The release water provided for this study was a product of adding gypsum ($CaSO_4$) to MFT. It was found that the gypsum treated water caused visible signs of phytotoxicity in both cattail and common reed. The gypsum treated release water also caused a significant reduction in the whole plant fresh weight and water uptake of cattail.

The results of this study can be used to compare the potential phytotoxicity of AFD materials with an older tailings technology. It appears as though both cattail and common reed are less sensitive to gypsum treated CT release water than AFD release water. However, both species were able to grow in AFD runoff water. The long term intention is to place dewatered AFD tailings in mined-out pits, which would then be covered with overburden. The release water is intended for recycling back into the extraction process. These studies give an indication that in the event of flooding due to heavy rain or spring snow melt, cattail and common reed used in reclamation strategies involving capped AFD deposits would be able to tolerate growing in AFD runoff water.

6.4.2 Nutrient media spiked with naphthenic acids

A hydroponic study was conducted to determine the phytotoxicity of naphthenic acids to cattail and common reed. As discussed in Section 1.3, oil sands tailings contain several sources of phytotoxicity including high salinity, high pH, and naphthenic acids. In order to determine the potential phytotoxicity of naphthenic acids alone, the naphthenic acids were extracted from MFT (Section 4.2.1) and this extract was used to spike hydroponic nutrient media (40 mg/L). The naphthenic acids significantly reduced the whole plant fresh weight and water uptake of cattail. Common reed proved to be able to grow in the nutrient media spiked with naphthenic acids. No mortality was observed for either species and neither species stopped taking up water, but instead reached a plateau in water uptake. The naphthenic acid concentration chosen for this study was the average concentration found in the AFD release water and MFT reclaim water collected. The results found here indicate that at the concentration evaluated, naphthenic acids alone are

not responsible for the phytotoxicity observed in hydroponic studies conducted with different tailings water types.

6.5 Naphthenic acids

Objective: *To determine the naphthenic acid molecular profile and fingerprint of AFD release and runoff waters in relation to MFT waters.*

Low resolution mass spectrometry was used to determine the naphthenic acid molecular profile for Shell oil sands tailings materials, which to the author's knowledge, had not yet been established. Using low resolution mass spectrometry, it was found that differences in the range of compounds present in the naphthenic acid mixture from Shell MFT and Shell AFD release water could not be detected. High resolution analyses provided further evidence of plant mediated changes to the naphthenic acid mixtures. Small changes detected using high-resolution mass spectrometry may be explained by a combination of microbial biodegradation and the dissipation of some compounds by cattail. It does not appear that common reed is able to dissipate naphthenic acids, but may however be releasing compounds as part of a phytotoxic response.

6.6 Future research opportunities

The work conducted for this project indicated knowledge gaps that require further investigation:

1) Longer and larger field scale hydroponic studies are needed to help determine potential phytotoxic effects of chronic exposure to waters associated with oil sands tailings. The 30

day laboratory studies conducted only provided a glimpse into the acute phytotoxic effects. It appears as though the aquatic macrophytes studied, particularly common reed, were beginning to adapt to growing in oil sands tailings water. Longer studies would provide insight into the long term effects of the oil sands tailings water on the life cycles of the plants, including the ways in which the plants may adapt. A shift from laboratory studies to field studies would provide insight into whether these species are suitable for reclamation, as well as if one species is more adaptable than the other.

For the present research, longer laboratory studies were considered, however at the end of the 30 day studies, the plants (cattail in particular) were becoming too large for the glass jars used. A longer study period could be evaluated, but the researcher would need to find a larger vessel to house the plants.

2) Multi-season collection of runoff water from the AFD deposits is needed to determine the effects of aging on dewatered AFD tailings. Analysis of multi-season runoff water would give an indication if over time, the polyacrylamide polymer added to the tailings is being degraded into acrylamide monomers. Water collected from the beginning of the spring, through to the end of the spring runoff would also give a more realistic indication of the fluctuations in pollutant load during the spring thaw. Use of water collected throughout the spring thaw in hydroponic studies would provide information on whether the initial water flowing from the AFD deposit is acutely phytotoxic.

3) Further analysis of the compounds released by aquatic plants studied in response to phytotoxic stress would give an indication of whether the changes seen in the

composition of the naphthenic acid mixture is due to plant mediated changes or biodegradation. Due to the complexity of the naphthenic acid mixture, it is difficult to determine which constituents are part of the naphthenic acids, and which constituents are the results of dissipation and/or biodegradation. As analytical techniques improve, more and more of the compounds collectively termed ‘naphthenic acids’ will be identified, allowing researchers to distinguish between plant fatty acids, products of microbial biodegradation, and any other compounds that may be present in the complex mixture.

REFERENCES

Allen EW. 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *Journal of Environmental Engineering and Science,* 7:123-138.

American Association of Petroleum Geologists. 2011. Unconventional energy resources: 2011 Review. *Natural Resources Research,* 20:279-328.

Apostol K, Zwiazek J. 2003. Hypoxia affects root sodium and chloride concentrations and alters water conductance in salt-treated jack pine (*Pinus banksiana*) seedlings. *Trees,* 17:251-257.

Apostol K, Zwiazek J. 2004. Boron and water uptake in jack pine (*Pinus banksiana*) seedlings. *Environmental and Experimental Botany,* 51:145-153.

Armstrong SA. 2008. Dissipation and Phytotoxicity of Oil Sands Naphthenic Acids in Wetland Plants. Doctoral dissertation; University of Saskatchewan, Saskatoon, Saskatchewan, 2008. pp5.

Armstrong SA, Headley JV, Peru KM, Germida JJ. 2008. Phytotoxicity of oil sands naphthenic acids and dissipation from systems planted with emergent aquatic macrophytes. *Journal of Environmental Science and Health, Part A* 43:36-42.

Armstrong SA, Headley JV, Peru KM, Germida JJ. 2009. Differences in phytotoxicity and dissipation between ionized and nonionized oil sands naphthenic acids in wetland plants. *Environmental Toxicology and Chemistry*, 28:2167-2174.

Armstrong SA, Headley JV, Peru KM, Mikula RJ, Germida, JJ. 2010. Phytotoxicity and naphthenic acid dissipation from oil sands fine tailings treatments planted with the

emergent macrophyte *Phragmites australis*. *Journal of Environmental Science and Health Part A*, 45:1008-1016.

Barrow MP, Witt M, Headley JV, Peru KM. 2010. Athabasca oil sands process water: characterization by atmospheric pressure photoionization and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Analytical Chemistry*, 82:3727-3735.

Barvenik FW. 1994. Polyacrylamide characteristics related to soil applications. *Soil Sciences*, 158:235-243.

Bendell-Young LI, Bennett KE, Crowe A, Kennedy CJ, Kermode AR, Moore MM, Plant AL, Wood A. 2000. Ecological characteristics of wetlands receiving an industrial effluent. *Ecological Applications*, 10:310-322.

Biryukova OV, Fedorak PM, Quidean SA. 2007. Biodegradation of naphthenic acids by rhizosphere microorganisms. *Chemosphere*, 67:2058-2064.

Brough S, Riley S, McGrady G, Tanhawiriyakul S, Romero-Zeron L, Wilson C. 2010. Low temperature extraction and upgrading of oil sands and bitumen in supercirtical fluid mixtures. *Chemical Communications*, 46:4923-4925.

Carbonell AA, Aarabi MA, DeLaunce RD, Gambrell RP, Patrick Jr WH. 1998. Arsenic in wetland vegetation: Availability, phytotoxicity, uptake, and effects on plant growth and nutrition. *Science of the Total Environment*, 217:189-199.

Clemente JS, Fedorak PM. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere*, 60:585-600.

Clemente JS, MacKinnon MD, Fedorak PM. 2004. Aerobic biodegradation of two commercial naphthenic acids preparations. *Environmental Science and Technology*, 38:1009-1016.

Colbeck, SC. 1981. A simulation of the enrichment of atmospheric pollutants in snow cover runoff. *Water Resources Research*, 17:1383-1388.

Crowe A, Han B, Kermode A, Bendell-Young L, Plant A. 2001. Effects of oil sands effluent on cattail and clover: photosynthesis and level of stress proteins. *Environmental Pollution*, 113:311-322.

Crowe A, Plant A, Kermode A. 2002. Effects of an industrial effluent on plant colonization and on the germination and post-germinative growth of seeds of terrestrial and aquatic plant species. *Environmental Pollution*, 117:179-189.

Demoz A, Mikula R. 2012. Role of mixing in the flocculation of mature fine tailings. *Journal of Environmental Engineering*, 138:129-136.

Doerge DR, Young JF, Chen JJ, DiNovi MJ, Henry SH. 2008. Using dietary exposure and physiologically based pharmacokinetic/pharmacodynamic modeling in human risk extrapolations for acrylamide toxicity. *Journal of Agricultural and Food Chemistry*, 56:6031-6038.

Energy Resources Conservation Board. 2009. ERCB Directive 074: Tailings Performance Criteria and Requirements for Oil Sands Mining Schemes. Calgary: ERCB.

Farwell A, Nero V, Ganshorn K, Leonhardt C, Ciborowski J, MacKinnon M, Dixon, DG. 2009. The use of stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) to trace exposure to oil sands processed material in the Alberta oil sands region. *Journal of Toxicology and Environmental Health*, 72:385-396.

Foote L, Hornung J. 2007. The growth and photosynthesis of typha in oil sands process affected material and water. *Proceedings of the 34th Annual Aquatic Toxicity Workshop*, Halifax Nova Scotia (2007).

Friedman M. 2003. Chemistry, biochemistry, and safety of acrylamide. A review. *Journal or Agricultural and Food Chemistry*, 51:4504-4526.

Gentes M, McNabb A, Waldner C, Smits J. 2007. Increased thyroid hormone levels in tree swallows (*Tachycineta bicolor*) on reclaimed wetlands of the Athabasca Oil Sands. *Archives of Environmental Contamination and Toxicology*, 53:287-292.

Grant J, Dagg J, Dyer S, Lemphers N. 2010. Northern Lifeblood. Empowering northern leaders to protect the Mackenzie River Basin from oil sands risks. The Pembina Institute. Available as PDF from www.pembina.org (assessed February 14th, 2012).

Gurney KE, Williams TD, Smits JE, Wayland M, Trudeau S, Bendell-Young LI. 2005. Impact of oil-sands based wetlands on the growth of mallard (*Anas platyrhynchos*) ducklings. *Environmental Toxicology and Chemistry*, 24:457-463.

Han X, Scott AC, Fedorak PM, Bataineh M, Martin JW. 2008. Influence of molecular structure on the biodegradability of naphthenic acids. *Environmental Science and Technology*, 42:1290-1295.

Harms J, Fairhurst GD, Bortolotti GR, Smits JEG. 2010. Variation in immune function, body condition, and feather corticosterone in nestling Tree Swallows (*Tachycineta bicolor*) on reclaimed wetlands in the Athabasca oil sands, Alberta, Canada. *Environmental Pollution*, 158:841-848.

Harris ML. 2007. Guidelines for wetland establishment on reclaimed oil sands leases (revised second edition). Prepared by Lorax Environmental for CEMA Wetlands and

Aquatics Subgroup of the Reclamation Working Group, Fort McMurray, AB. February 2007. Available online at www.environment.gov.ab.ca/info/library/8105.pdf.

Hazma H, Stanonik D, Kessick M. 1996. Flocculation of lime-treated oil sands tailings. *Fuel*, 75:280-284.

Headley JV, Peru KM, McMartin DW, Winkler M. 2002. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. *Journal of AOAC International*, 85:182-187.

Headley JV, McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances and Environmental Engineering*, 39: 1989-2010.

Headley JV, Peru KM, Barrow MP. 2009. Mass spectrometric characterization of naphthenic acids in environmental samples: A review. *Mass Spectrometry Reviews*, 28: 121-134.

Headley JV, Peru KM, Mishra S, Meda V, Dalai A, McMartin D, Mapolelo MM, Rodgers RP, Marshall, AG. 2010. Characterization of oil sands naphthenic acids treated with ultraviolet and microwave radiation by negative ion electrospray Fourier transform ion cyclotron resonace mass spectrometry. *Rapid Communications in Mass Spectrometry*, 24:3121-3126.

Headley JV, Barrow MP, Peru KM, Fahlman B, Frank RA, Bickerton G, McMaster ME, Parrott J, Hewitt LM. 2011. Preliminary fingerprinting of Athabasca oil sands polar organics in environmental samples using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Rapid Communications in Mass Spectrometry*, 25:1899-1909.

Headley JV, Barrow MP, Peru KM, Derrick PJ. 2011. Salting-out effects on the characterization of naphthenic acids from Athabasca oil sands using electrospray ionization. *Journal of Environmental Science and Health Part A*, 46-844-854.

Hersikorn BD, Ciborowski JJ, Smits JEG. 2010. The effects of oil sands wetlands on wood frogs. *Toxicological and Environmental Chemistry*, 92:1513-1527.

Hoagland DR, ARNON DI. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station*, 347:1-32.

Holowenko FM, MacKinnon MD, Fedorak PM. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Research*, 36:2843-2855.

Janfada A, Headley JV, Peru KM, Barbour SL. 2006. A laboratory evaluation of the sorption of oil sands naphthenic acids on organic rich soils. *Journal of Environmental Science and Health Part A*, 41:985-997.

Johannessen M and Henriksen A. 1987. Chemistry of snow meltwater: Changes in concentration during melting. *Water Resources Research*, 14:615-619.

Johnson E, Miyanishi K. 2008. Creating new landscapes and ecosystems: The Alberta oil sands. *Annals of the New York Academy of Sciences*, 1134:120-145.

Kannel PR, Gan TY. 2012. Naphthenic acids degradation and toxicity mitigation in tailings wastewater systems and aquatic environments: A review. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, 47:1-21.

Kasperski KL, Mikula RJ. 2011. Waste streams of mined oil sands: Characteristics and remediation. *Elements*, 387-392.

Kim S, Stanford LA, Rodgers RP, Marshall AG, Walters CC, Qian K, Wenger LM, Mankiewicz P. 2005. Microbial alteration of the acidic and neutral polar NSO compounds revealed by Fourier transform ion cyclotron resonance mass spectrometry. *Organic Geochemistry*, 36:1117-1134.

Krauth DM, Green VS, Baker WH, Bouldin JL, Wren PS. 2008. Evaluation of a polyacrylamide soil additive to reduce agricultural associated contamination. *Bulletin of Environmental Contamination and Toxicology*, 81:116-123.

Leung SS, MacKinnon M, Smith REH. 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. *Environmental Toxicology and Chemistry*, 20:1532-1543.

Lentz RD, Andrawes FF, Barvenik FW, Koehn AC. 2008. Acrylamide monomer leaching from polyacrylamide-treated irrigation furrows. *Journal of Environmental Quality*, 37:2293-2298.

Lister A, Nero V, Farwell A, Dixon DG, Van Der Kraak G. 2008. Reproductive and stress hormone levels in goldfish (*Carassius auratus*) exposed to oil sands process-affected water. *Aquatic Toxicology*, 87:170-177.

Long J, Li H, Xu Z, Masliyah J. 2005. Role of colloidal interactions in oil sands tailings treatment. *American Institute of Chemical Engineers*, 52:371-383.

Mackinnon MD, Elshayeb M, George D, Power M. 2009. The use of carbon and nitrogen stable isotope analysis to characterize food web changes in aquatic systems for reclamation of oil sands process-affected materials. *Water Quality Research Journal of Canada*, 44:313-322.

Mal T, Narine L. 2004. The biology of Canadian weeds. 129. *Phragmites australis* (Cav.) Trin. Ex Steud. *Canadian Journal of Plant Science*, 84:365-396.

McMartin DW, Headley JV, Friesen DA, Peru KM, Gillies JA. 2004. Photolysis of naphthenic acids in natural surface water. *Journal of Environmental Science and Health, Part A*, 39:1361-1383.

Michaud AM, Chappellaz C, Hinsinger P. 2008. Copper phytotoxicity affects root elongation and iron nutrition in durum wheat (*Triticum turgidum durum* L.). *Plant and Soil*, 310:151-165.

Mikula RJ, Munoz VA, Omotoso O. 2008. Water use in bitumen production: Tailings management in surface mined oil sands, in *Proceedings 2nd World Heavy Oil Conference*, paper 436, Edmonton, Alberta, 2008.

Mitich L. (2000). Common Cattail, *Typha latifolia* L. *Weed Technology*, 14, 446-450.

Nero V, Farwell A, Lister A, Van Der Kraak G, Lee LEJ, Van Meer T, MacKinnon MD, Dixon DG. 2006. Gill and liver histopathological changes in yellow perch (*Perca flavescens*) and goldfish (*Carassius auratus*) exposed to oil sands process-affected water. *Ecotoxicology and Environmental Safety*, 63:365-377.

Oil Sands Wetlands Working Group. 2000. Guideline for wetland establishment on reclaimed oil sands leases. Alberta: Alberta Environment.

Penner T, Foght J. 2010. Mature fine tailings from oil sands processing harbour diverse methanogenic communities. *Canadian Journal of Microbiology*, 56:459-470.

Pollet I, Bendell-Young LI. 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. *Environmental Toxicology and Chemistry*, 19:2589-2597.

Quagraine EK, Peterson HG, Headley JV. 2005. In situ bioremediation of naphthenic acids contaminated tailing pond waters in the Athabasca Oil Sands Region – demonstrated field studies and plausible options: a review. *Journal of Environmental Science and Health, Part A*, 40:685-722.

Radio Netherlands Worldwide, 2012. Photograph available at www.rnl.nl, accessed September 30, 2012.

Redfield E, Croser C, Zwiazek JJ, MacKinnon MD, Qualizza C. 2003. Responses of red-osier dogwood to oil sands tailings treated with gypsum or alum. *Journal of Environmental Quality*, 32:1008-1014.

Renault S, Lait C, Zwiazek JJ, MacKinnon M. 1998. Effect of high salinity tailings waters produced from gypsum treatment of oil sands tailings on plants of the boreal forest. *Environmental Pollution*, 102:177-184.

Renault S, Paton E, Nilsson G, Zwiazek J, MacKinnon M. 1999. Responses of boreal plants to high salinity oil sands tailings water. *Journal of Environmental Quality*, 128:1957-1962.

Renault S. Zwiazek JJ, Fung M, Tuttle S. 2000. Germination, growth and gas exchange of selected boreal forest seedlings in soil containing oil sands tailings. *Environmental Pollution*, 107:357-365.

Renault S, MacKinnon M, Qualizza C. 2003. Barley, a potential species for initial reclamation of saline composite tailings of oil sands. *Journal of Environmental Quality*, 32:2245-2253.

Renaullt S, Qualizza C, MacKinnon M. 2004. Suitability of altai wildrye (*Elymus angustus*) and slender wheatgrass (*Agropyron trachycaulum*) for initial reclamation of saline composite tailings of oil sands. *Environmental Pollution*, 128:339-349.

Rogers SD, Beech J, Sarma KS. 1998. Shoot regeneration and plant acclimatization of the wetland monocot Cattail (*Typha latifolia*). *Plant Cell Reports*, 18:71-75.

Rogers VV, Liber K, MacKinnon MD. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. *Chemosphere*, 48:519-527.

Rooney RC, Bayley SE. 2011. Setting reclamation targets and evaluating progress: Submerged aquatic vegetation in natural and post-oil sands mining wetlands in Alberta, Canada. *Ecological Engineering*, 37:569-579.

Ross MS, dos Santos Pereira A, Fennell J, Davies M, Johnson J, Sliva L, Martin JW. 2012. Quantitative and qualitative analysis of naphthenic acids in natural waters surrounding the Canadian oil sands industry. *Environmental Science and Technology*, 46:12796-12805.

Scott AC, MacKinnon MD, Fedorak PM. 2005. Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. *Environmental Science and Technology*, 39:8388-8394.

Shell Canada Energy. 2010. Muskeg River Mine Atmospheric Fines Drying Phase I – 250 K Tonne Fines Capture Test. ERCB Directive 074 Submission. June 2010. Available for download at: www.ercb.ca/regulations-and-directives/directive074/tailings-2009

Smith EA, Prues SL, Oehme FW. 1996. Environmental degradation of polyacrylamides. 1. Effects of artificial environmental conditions: Temperature, light, and pH. *Ecotoxicology and Environmental Safety*, 35:121-135.

Smith EA, Prues SL, Oehme FW. 1997. Environmental degradation of polyacrylamides. II. Effects of environmental (outdoor) exposure. *Ecotoxicology and Environmental Safety*, 37:76-91.

Tatarniuk C, Donahue R, Sego D. 2009. Snow characterization at a city snow storage facility. *Journal of Cold Regions Engineering*, 23:136-142.

Teodorović I, Knežević V, Tunić T, Čučak M, Nikolić Lečić J, Leovac A, Ivančev Tumbas I. 2012. *Myriophyllum aquaticum* versus *Lemna minor*: Sensitivity and recovery potential after exposure to atrazine. *Environmental Toxicology and Chemistry*, 31:471-426.

Trites M, Bayley S. 2009. Vegetation communities in continental boreal wetlands along a salinity gradient: Implications for oil sands mining reclamation. *Aquatic Botany,* 91:27-39.

United States Department of Agriculture. 2010. Retrieved October 6th, 2010, from Natural Resources Conservation Service: http://plants.usda.gov

van den Heuvel MR, Power M, MacKinnon MD, Van Meer T, Dobson EP, Dixon DG 1999. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). I. Water quality characteristics and yellow perch physiological and population responses. *Canadian Journal of Fisheries and Aquatic Sciences*, 56:1213-1225.

van den Heuvel MR, Power M, MacKinnon MD, Dixon DG. 1999. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). II. Chemical and biochemical indicators of exposure to oil sands related waters. *Canadian Journal of Fisheries and Aquatic Sciences*, 56:1226-1233.

Wang X, Feng, X, Xu Z, Masliyah, J. 2010. Polymer aids for settling and filtration of oil sands tailings. *The Canadian Journal of Chemical Engineering*, 88:403-410.

West, CE, Jones, DJ, Scarlett, AG, Rowland, SJ. 2011. Compositional heterogeneity may limit the usefulness of some commercial naphthenic acids for toxicity assays. *Science of the Total Environment*, 409:4125-4131.

Wort DJ, Severson Jr. JG, Peirson DR. 1973. Mechanism of plant growth stimulation by naphthenic acid: growth stimulation of *Phaseolus vulgaris* L. *Plant Physiology*, 52:162-165.

Xu Y, Dabros T, Kan J. 2008. Filterability of oil sands tailings. *Process Safety and Environment Protection*, 86:268-276.

Yuan X, Shaw W. 2007. Novel processes for treatment of Syncrude fine transition and marine or tailings. *Canadian Metallurgical Quarterly*, 46:265-272.

APPENDIX A – Water chemistry data

Table A.1. Water chemistry parameters measured in oil sands tailings water samples collected from tailings provided for the use in the hydroponic phytotoxicity experiments.

Inorganic Chemistry Data	MFT Reclaim	Cell 8 Release	Composite Release	Cell 4 Runoff	Cell 7 Runoff	Cell 8 Runoff	MFT w/Gypsum	MFT w/out Gypsum
Bicarbonate (mg/L)	460	570	603	439	289	556	886	525
Carbonate (mg/L)	10	29	11	4	4	5	<1	7
Chloride (mg/L)	220	2920	285	178	148	311	427	558
Hydroxide (mg/L)	<1	<1	<1	<1	<1	<1	<1	<1
P. Alkalinity (mg/L)	8	24	9	3	3	4	<1	6
pH	8.44	8.61	8.43	8.38	8.39	8.48	8.13	8.16
Specific Conductivity (µS/cm)	1660	1910	1880	1340	1180	1920	3280	3050
Sum of Ions (mg/L)	1240	1420	1440	1030	857	1420	2480	2030
Total Alkalinity (mg/L)	393	515	512	366	243	464	7.26	442
Total Hardness (mg/L)	107	118	131	173	112	129	116	75
Nitrate (mg/L)	0.75	13	15	0.56	1.2	1.1	53	71
ICP-MS Data								
Calcium (mg/L)	23	26	31	43	30	32	25	12
Magnesium (mg/L)	120	13	13	16	9	12	13	11
Potassium (mg/L)	18	15	15	10	6.9	12	18	24
Sodium (mg/L)	314	369	362	227	209	373	697	669
Sulfate (mg/L)	180	95	100	110	160	120	360	150

APPENDIX B – Instrument operating parameters for naphthenic acid analysis using low resolution mass spectrometry

Analyses of samples for this thesis were performed using electrospray ionization mass spectrometry (ESI-MS) (Headley *et al*., 2002). Mass spectrometric analysis was conducted using a Quattro Ultima mass spectrometer (Waters/Micromass, UK) equipped with an electrospray interface operating in the negative ion mode. Mass spectrometer conditions for analysis of the mixtures were set as follows: source temperature 90ºC, desolvation temperature 220ºC, cone voltage setting 62 V, capillary voltage setting 2.63 kV, cone gas N_2 158 L/h, desolvation gas N_2 489 L/h. The multiplier was set at 650 V. Full scan MS was employed in the m/z range 50 - 550. Samples (5 µL) were loop injected using a Waters 2695 (Waters Corp) Separations Module using 50:50 acetonitrile:water containing 0.4% ammonium hydroxide as the eluent at 200 µL/min.

APPENDIX C – Instrument operating parameters for naphthenic acid analysis using high resolution mass spectrometry

Analysis of sample extracts for this thesis was performed on a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) using electrospray ionization in negative ion mode. Electrospray ionization source conditions were as follows: heater temperature was set to 50°C, sheath gas flow rate was set to 25 (arbitrary units), auxiliary gas flow rate was set to 5 (arbitrary units), spray voltage set to 2.90 kV, capillary temperature was set to 275°C and the S lens RF level was set to 67%. Samples were analyzed in full scan with an m/z range of 100-600, resolution was set to 100,000. Resulting naphthenic acid concentrations were determined by comparison to a pre-defined 5-point regression of naphthenic acids at known concentrations. Xcalibur version 2.1 software (Thermo Fisher Scientific) was used for data acquisition, instrument operation and quantitative data analysis. Class distribution was determined using acquired accurate mass data and Composer version 1.0.2 (Sierra Analytics, Inc.).